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HOSTS OF THE TOBACCO STREAK VIRUS[1]

ROBERT W. FULTON

(Accepted for publication October 13, 1947)

The tobacco streak disease was first described, from Wisconsin, by Johnson (7). He reported it in 1935 as usually occurring on scattered plants, having seen it in abundance only once. Since then it has become much more common and has been reported from other states and countries. It is widely distributed in Wisconsin; by the middle of early August nearly every field of tobacco has at least a few infected plants. A high percentage of infection along the borders of fields is not uncommon.

In order to devise control methods, information is needed on the manner in which the virus overwinters and how it spreads to tobacco. The localization of the disease near the edges of tobacco fields led Johnson (7) to suggest that infected perennial weeds were the source of the virus. Except for the experimental infection of 35 species of *Nicotiana* by Diachun and Valleau (6), only a few species in other genera are known to be susceptible to the virus. Anderson (2) reported the infection of jimson weed and ground cherry and thought that the virus probably was transmitted from these hosts to tobacco. A high incidence of tobacco streak near perennial sweetclover has been reported by Valleau (8) and Berkeley and Phillips (4). In Wisconsin, none of the localized areas of heavily infected tobacco has been found adjacent to sweetclover. For this reason, other plants were suspected of harboring the virus. The present paper reports various plant species found infected near tobacco fields, and lists species found susceptible by artificial inoculation.

METHODS AND MATERIALS

To determine the incidence of the streak virus in wild and crop plants, collections were made in areas near infected tobacco fields. Young leaves of plants showing virus-like symptoms, as well as others appearing healthy, were macerated in a mortar and the extract wiped with a gauze pad on the leaves of 5 small healthy Havana-Seed tobacco plants in the greenhouse. The tobacco leaves were dusted lightly with carborundum before being inoculated, and rinsed with water after inoculation. These plants were observed for two weeks or longer under conditions favorable for the development of streak symptoms.

In determining the experimental host range of the virus, species[2] of plants, selected at random, were grown from seed in the greenhouse. When

[1] Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

[2] The author wishes to thank the Seed Inspection and Weed Control Laboratory, Wisconsin Department of Agriculture, for identifying certain species, and for providing seed of others.

still young, the plants were dusted with carborundum and inoculated with a fresh extract of the streak virus from recently infected tobacco plants. After 10 to 14 days, an attempt was made to transfer the virus from these plants to 5 healthy tobacco plants. If no infection resulted, the original plants were reinoculated and a second effort was made to recover the virus on tobacco.

NATURAL INFECTION IN WEEDS AND CROP PLANTS

In the late summer and fall of 1946, 159 inoculations were made from various species collected on 6 farms, on 5 of which tobacco streak was

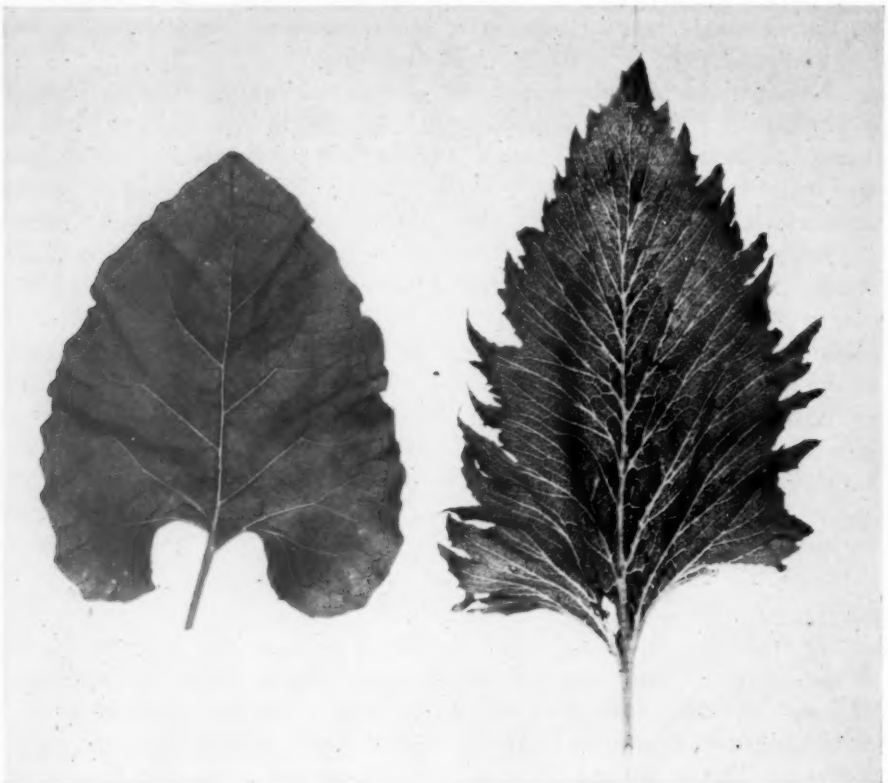

FIG. 1. Leaves of burdock (*Arctium minus*) showing the toothed type of malformation due to infection with the tobacco streak virus. The leaf on the left is healthy; the leaf on the right is from a naturally infected plant.

abundant. Nine of these inoculations produced typical tobacco streak on tobacco. Table 1 shows the species found infected and the number of times that the virus was obtained from each. As a check on the identity of the viruses isolated, it was shown that "recovered" leaves of the infected tobacco plants did not develop new symptoms when inoculated with known tobacco streak virus.

The symptoms on these naturally infected plants are inconspicuous,

except on burdock (*Arctium minus* (Hill) Bernh.). The leaves of infected burdock plants are strikingly toothed and thickened, with prominent light colored veins (Fig. 1). The infected plants appear very similar to an anonymous description (1) of *Arctium minus* form *laciniatum*. This taxonomic form may have been based on plants infected with the streak virus.

The survey was continued in the spring of 1947 in the same localities. Tobacco streak was recovered 12 times in 127 inoculations from various plants. The virus was most consistently isolated from burdock (Table 1).

TABLE 1.—*Plant species collected during 2 seasons and found to be naturally infected with the tobacco streak virus*

Species	Farm	Collected fall, 1946		Collected spring, 1947	
		Plants tested	Plants infected	Plants tested	Plants infected
		Number	*Number*	*Number*	*Number*
Burdock	A	9	2	7	3
(*Arctium minus*	B	3	0	3	2
(Hill) Bernh.)	C	1	0	.....	.....
	D	.....	.....	3	2
	E	.....	.....	5	0
	F	.....	.....	2	1
Hedge Mustard	A	5	3	.....	.....
(*Sisymbrium*	B	1	1	.....	.....
officinale (L.)	D	1	0	.....	.....
Scop.)					
White Clover	A	3	2	4	1
(*Trifolium*	C	4	0	.....	.....
repens L.)	D	.....	.....	4	0
	E	1	0	1	0
	F	1	0	1	0
Bindweed	A	4	1	.....	.....
(*Convolvulus*					
arvensis L.)					
Plantain	A	1	0	4	0
(*Plantago*	D	2	0	1	1
major L.)	E	1	0	3	0
	F	.....	.....	3	0

This may have been due to the conspicuous symptoms, which made it easy to recognize infected plants. On two occasions, not listed in table 1, infected burdock plants were found some distance away from tobacco fields. The virus was never recovered from burdock showing mosaic, yellowing, necrosis, or symptoms other than the toothed type of malformation. Burdock, in its perennial habit and its wide and abundant distribution, fulfills all the requirements for an overwintering host of the streak virus.

Twenty-four inoculations were made from sweetclover showing various virus-like symptoms. None of these produced tobacco streak. Valleau (8) and Berkeley and Phillips (4) have reported difficulty in mechanically

transmitting this virus from sweetclover to tobacco. For this reason, negative evidence may be of little significance. The virus could be transmitted readily, however, from artificially infected sweetclover in the greenhouse, indicating that at least some infection might have been obtained from field grown plants if they had contained the virus.

EXPERIMENTAL HOST RANGE

Since the species of plants found naturally infected, including tobacco, represented 6 different families, it seemed likely that the virus might be able to infect many others. In greenhouse tests, this proved to be true. Of the 169 species selected at random and inoculated, the virus was subsequently recovered on tobacco from the following 87 species, listed by families. Species in parentheses were not tested but are listed because they have been reported susceptible by other authors.

APOCYNACEAE
Vinca rosea L.

ASCLEPIADACEAE
Asclepias syriaca L.

CARYOPHYLLACEAE
Cerastium vulgatum L.
Lychnis alba Mill.
Silene noctiflora L.
Spergula arvensis L.

CHENOPODIACEAE
Beta vulgaris L.
Chenopodium album L.
Spinacia oleracea L.

COMPOSITAE
Arctium minus (Hill) Bernh.
Callestephus chinensis Nees.[3]
(*Calendula officinalis* L.)[4]
Ambrosia artemisifolia L.
A. trifida L.
Cichorium endiva L.
Taraxacum officinale Weber
Sonchus oleraceus L.
Lactuca sativa L.
Venidium fastuosum
Zinnia elegans Jacq.

CONVOLVULACEAE
Convolvulus arvensis L.
Convolvulus sp.

CRUCIFERAE
Brassica arvensis L.
Iodanthus pinnatifidus (Michx.) Steud.
Sisymbrium officinale (L.) Scop.

CUCURBITACEAE
Cucumis sativus L.
C. melo L.
Cucurbita maxima Duchesne.
C. pepo L.
Citrullus vulgaris Schrad.
Sicyos angulatus L.

LABIATAE
Nepeta cataria L.

LEGUMINOSAE
Aeschynomene indica L.
Crotalaria intermedia Kotschy
Cyamopsis tetragonalobus (L.) Taub.
Dolichos biflorus L.
Hedysarum coronarium L.
Melilotus alba Desr.
M. officinalis Lam.
Phaseolus aconitifolius Jacq.
P. aureus Roxb.
P. vulgaris L.[4]
Pisum sativum L.
Psoralea bituminosa L.
Robinia pseudo-acacia L.
Soya max (L.) Piper.
Trifolium glomeratum L.
T. incarnatum L.
T. praetense L.
T. repens L.
Trigonella sp.
Vicia faba L.

MALVACEAE
Abutilon theophrasti Medic.
Althaea rosea L.
Hibiscus esculentus L.
Hibiscus sp.
Gossypium hirsutum L.
Malva rotundifolia L.

NYCTAGINACEAE
Mirabilis jalapa L.

ONAGRACEAE
Oenothera biennis L.

PLANTAGINACEAE
Plantago major L.

POLYGONACEAE
Fagopyrum esculentum Gaertn.

Rumex britannica L.
R. crispus L.
Polygonum persicarae L.

PORTULACACEAE

Portulaca oleracea L.

ROSACEAE

Fragaria virginiana Duchesne.
Geum sp.
Potentilla monspeliensis L.

SOLANACEAE

Capsicum frutescens L.[3]
Datura metel L.
D. stramonium L.[4, 5, 6]
Lycium halimifolium Mill.
Lycopersicon esculentum Mill.[7, 8]
Nicandra physaloides (L.) Pers.[4, 5]
Nicotiana spp.
Petunia hybrida Vilm.[3]
Physalis pubescens L.[5, 6]
Schizanthus wisetonensis Low.
Solanum aculeatissimum Jaeq.
S. dulcamara L.
S. integrifolium Poir.
S. melongena L.[7]
S. nigrum L.
S. triflorum Nutt.
S. tuberosum L.[7, 8]

SCROPHULARIACEAE

(*Antirrhinum majus* L.)[4]
Verbascum thapsus L.

TROPAEOLACEAE

Tropaeolum majus L.

UMBELLIFERAE

Apium graveolans L.

Under high greenhouse temperatures (80° F.) and short-day conditions, the following symptoms were characteristic of the families involved: *COMPOSITAE*, mosaic or necrotic flecking; *CUCURBITACEAE*, yellow mottle; *LEGUMINOSAE*, necrosis, slight mottle, or symptomless; *MALVACEAE*, brown necrotic spots and lines; *ROSACEAE*, mosaic and stunting; *SOLANACEAE*, chlorotic ring spot, mosaic, or systemic necrotic lines. On most of the other species the symptoms were not distinctive.

All of the species listed were infected by mechanical inoculation except *Pisum sativum* L. and *Trifolium praetense* L. These were infected through a dodder (*Cuscuta campestris* L.) connection to diseased tobacco, the virus being transferred mechanically back to tobacco after removal of the dodder.

Ring-spot symptoms were produced on *Phytolacca decandra* L., and necrotic flecking on *Cichorium Intybus* L., but no virus could be recovered from these species either mechanically or through dodder.

The infection of 3 rosaceous species, and the subsequent recovery of the virus on tobacco, are of some interest. Bawden and Kleczkowski (3) have pointed out that no virus infecting the Rosaceae is known to be transmitted mechanically. They have suggested that this is due to virus inactivation by tannin when the infected leaves are crushed. Apparently this is not the case with the tobacco streak virus. Thirty-four of 40 tobacco plants became infected when inoculated with leaf extracts from infected Rosaceae, but only 12 of 44 rosaceous plants became infected when inoculated with the streak virus from tobacco. The limiting factor here appeared to be the relative insusceptibility of the 3 species.

A number of other species also showed a high degree of resistance. None of the cruciferous species could be infected consistently, and several solan-

[3] Previously reported not susceptible by Berkeley and Phillips (4).
[4] Previously reported susceptible by Berkeley and Phillips (4).
[5] Previously reported susceptible by Johnson (7).
[6] Previously reported susceptible by Anderson (2).
[7] Previously reported not susceptible by Johnson (7).
[8] Previously reported susceptible by Valleau (9).

aceous species, particularly tomato and potato, could not be infected regularly.

The species which did not become infected are not listed, since in some cases only a few plants were inoculated. It is likely that at least some of these would prove susceptible if larger numbers were used.

QUANTITATIVE ESTIMATION OF VIRUS INFECTIVITY

One of the purposes of inoculating a large number of species was to find a host more suitable than tobacco for certain types of research. A species

FIG. 2. Local lesions produced by the tobacco streak virus on guar (*Cyamopsis tetragonalobus*). The leaf was inoculated with a 1:1 dilution of the streak virus, and photographed 3 days after inoculation. The scale shown is in centimeters.

which develops local lesions would be particularly useful. Some varieties of garden beans developed the diffuse, reddish-brown, necrotic spots described by Berkeley and Phillips (4). Others, principally selections of the Great Northern variety, developed distinct necrotic spots. The number of lesions was small, and occasionally no infection could be obtained. Another legume, guar (*Cyamopsis tetragonalobus* (L.) Taub.), was much more susceptible than bean. Small, dark, local lesions appeared about 48 hours after inoculation (Fig. 2). These showed little tendency to enlarge or spread, remaining distinct for 10 days or more. The first 3 or 4 leaves the plant produces are simple and usually 5 to 8 cm. long. Although the leaves are not as large as the primary leaves of bean, the lesions were small, and several hundred on one leaf could be accurately counted.

Since an inoculum of high infectivity is necessary for consistent infection, some measurements of virus infectivity on guar are presented. As has been reported by Diachun (5), the presence of M/10 Na_2HPO_4 in the inoculum increased the number of lesions. It was found further that the amount of infective virus in tobacco was much greater in the inoculated leaves than in other parts of the plant. Comparisons of infectivity were made using inocula prepared by grinding 0.1 gram of tissue in 0.4 ml. of M/10 Na_2HPO_4. Three different extracts were inoculated in pairs on opposite half-leaves of guar, so that each possible pair occurred once on each of 3 leaf positions. Thus each extract was inoculated to a total of 6 half-leaves on 3 guar plants. In one trial, with inoculum taken from a tobacco plant which had been infected for 10 days, the extract of the inoculated tobacco leaves gave a total of 132 lesions on 6 half-leaves of guar, while extracts of the systemically diseased, and the symptomless tobacco leaves gave totals of 5 and 1 lesions, respectively. Other trials gave similar results.

It was also found that a marked decrease in infectivity occurred in extracts within a few minutes after the infected tissue was ground. In one representative trial, the left halves of 3 leaves on 3 plants were inoculated immediately after grinding 0.1 gram of tissue in 0.9 ml. of M/10 Na_2HPO_4. The right halves were inoculated, in a Latin square design, after 5, 10, and 15 minutes. The leaves inoculated immediately gave an average of 95 lesions on 3 half-leaves. The total lesions on the 3 half-leaves inoculated after 5, 10, and 15 minutes were 88, 62, and 31 respectively. Other trials, extending over longer periods, showed a greater decrease in the number of lesions. In order to obtain highly infective inoculum, therefore, tobacco leaves which had been inoculated 5 to 10 days previously were ground with M/10 Na_2HPO_4 and used immediately. Many of the host plants could be infected only when this practice was followed.

DISCUSSION

The recovery of the tobacco streak virus from various species near tobacco fields in the late summer, and again the following spring, indicates the manner in which the virus overwinters. In view of the wide host range, there undoubtedly were other species infected which were not found. The evidence indicates that burdock is an important reservoir for the streak virus in Wisconsin. It may be that in other regions different species are more commonly infected.

The relative importance of the different susceptible species to the streak disease of tobacco is difficult to determine. In the absence of a known insect vector, the actual spread of the virus from a wild host remains to be demonstrated. The habits and feeding preferences of the vector will determine, to some extent, which host plants are most important to the disease on tobacco.

The evidence suggests that the disease is endemic in certain wild plants. It is difficult, however, to account for the increasing amount of the disease on tobacco. Each of the 5 species found naturally infected is common and widely distributed. The virus is evidently not limited in its distribution by a lack of susceptible hosts.

SUMMARY

The following plants were found naturally infected with the tobacco streak virus in the vicinity of diseased tobacco fields: Burdock, white clover, hedge mustard, bindweed, and plantain. Burdock appears to be the weed which is most commonly infected with streak in Wisconsin.

A large experimental host range was found for the virus, 87 out of the 169 species inoculated being susceptible. Inoculum prepared from previously inoculated tobacco leaves was much more infective than inoculum from systemically diseased, or upper, symptomless tobacco leaves. The infectivity of the inoculum decreased rapidly within 15 minutes after the tissue was ground.

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THE YELLOW-NET VIRUS DISEASE OF SUGAR BEETS[1]

EDWARD S. SYLVESTER [2]

(Accepted for publication January 13, 1948)

INTRODUCTION

An apparently undescribed virus disease of the sugar beet (*Beta vulgaris* L.), causing a severe veinal chlorosis (Fig. 1, right) and transmissible by the green peach aphid, *Myzus persicae* (Sulzer) was collected in the field in June, 1945.[3] The virus is not juice transmissible, but can be transmitted with ease by means of the vector. The aphid retains the ability to transmit the virus throughout its experimental life, and consequently the virus is considered to be a persistent aphid-borne virus (24).

REVIEW OF LITERATURE

The previously described virus diseases of sugar beets are: curly top (21), Argentine curly top (2), sugar beet leaf-crinkle (26), savoy (4), beet mosaic (9), beet virus yellows (10), sugar-beet yellow vein (1), yellow wilt of sugar beets (3), and cucumber mosaic in sugar beets (9).

The yellow-net disease differs from all of these diseases in its syndrome. In addition, it varies from curly top, Argentine curly top, beet leaf-crinkle, savoy, yellow vein, and yellow wilt in that it can be transmitted by an aphid.

The three additional aphid transmissible virus diseases of sugar beets, *i.e.*, beet mosaic, beet yellows, and cucumber mosaic, have been recognized in the literature for a number of years. The sugar beet mosaic disease is world wide in its distribution (7, 11, 13, 14, 16). This virus is juice transmissible and is not retained for more than a few hours by its vector (23). The symptoms consist of an initial vein-translucency or yellowing on the youngest leaves. This is succeeded by a diffuse light and dark green mottling over the entire plant. Initial mild leaf distortion may occur in severe cases. In mature leaves the symptom expressed is usually one of small light green rings on a dark green background. The early stage of vein yellowing in some of the mosaic specimens often is indistinguishable from that produced by the yellow-net virus. However, the later phases of the two diseases are distinct in symptomatology.

Sugar beets are susceptible to the cucumber mosaic virus. This virus, like the beet mosaic virus, is juice transmissible, and is not retained by the aphid vectors.

[1] A condensed portion of a thesis presented to the graduate division of the University of California, February, 1947, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[3] The material was collected by L. D. Leach, Associate Plant Pathologist, University of California, Davis, California.

The symptoms induced in sugar beets by the cucumber mosaic virus are local lesions on inoculated leaves. These lesions are yellow, have diffuse margins, and tend to enlarge as the leaf ages. In systemic infection, the newly developing leaves are affected with a partial chlorosis, often apical, which is not confined by tissue regions. There is no vein translucency or vein yellowing in the newly developing leaves. Later the leaves develop a coarse green and yellow mottle, and at times the green areas cup outward to produce blister-like elevations. There is stunting and leaf malformation. In symptomatology, juice transmissibility, and vector relationships, this virus disease differs clearly from the yellow-net disease.

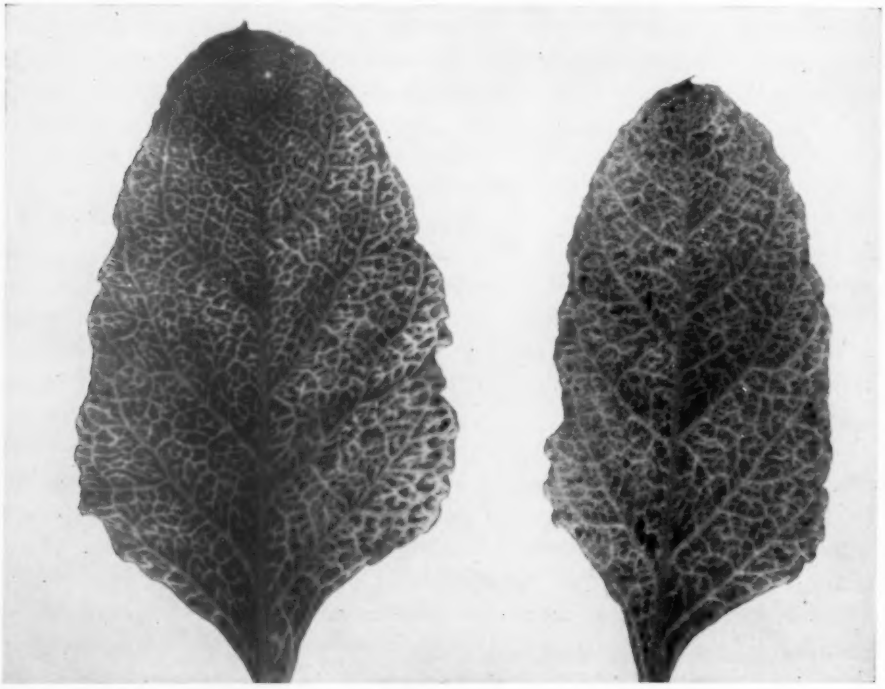

FIG. 1. Leaves from sugar beets experimentally infected with the yellow-net virus showing the chlorosis of the veins and veinlets.

The beet yellows virus and the yellow-net virus have two characteristics in common. They are transmitted by aphids and they are not juice inoculable. Beet yellows has been noted in England (14) and Europe (16), but not in the United States. Symptomatologically, the two diseases are distinct. Hull and Watson (10) reported the symptoms of beet yellows as consisting of a yellowing, thickening, and a brittleness of the outer or middle-aged leaves of the affected plant. The young central leaves generally remain green, and under favorable growing conditions they make moderate growth. Usually, however, in an early infection, growth almost ceases. The yellowed leaves later become necrotic and die. The symptoms

produced by the yellows virus are distinct from those induced by the yellow-net virus, and it is unlikely that any confusion between the two diseases would arise.

Bennett (1) made note of a disease of sugar beets which he termed sugar-beet yellow vein. He stated that the virus disease had not been described to date, but had been known to occur since 1936 in experimental plots at Riverside, California. It also had been known to occur in Colorado. The symptoms reported consisted of a "distinct yellowing along the main veins of leaves, dwarfing of the plant (often more marked on one side), and general yellowing of the foliage." Bennett also stated that the virus was not juice transmissible, but had been transmitted by grafting, and "apparently the virus is not transmissible by the aphid, *Myzus persicae* (Sulz.)."

The author has discussed the symptoms of the sugar-beet yellow vein disease with Bennett and has seen photographs of diseased plants. From the symptoms expressed on the photographed leaf blades, it would be difficult to distinguish the disease produced by the sugar-beet yellow vein virus and that induced by the yellow-net virus. However, the stunting, often asymmetrical, exhibited by plants infected with the sugar-beet yellow vein virus has never been observed in connection with the yellow-net virus. Furthermore, the latter virus is readily transmitted by the green peach aphid.

In spite of the similarity of the leaf symptoms of the two diseases, it is considered that the presence of asymmetrical stunting and lack of aphid transmission in the yellow vein disease described by Bennett is of sufficient importance to permit separation of that disease from the yellow net disease described in this paper.

MATERIALS AND METHODS

The original diseased plants were obtained on Sherman Island near Rio Vista, California. In later experiments, plants inoculated from these original plants were used as virus sources.

Noninfective aphid colonies were maintained upon large healthy sugar beets and originated from a colony of green peach aphid feeding upon a disease-free beet plant in the greenhouse.

The test sugar beet plants were grown from seed which had been planted in flats and later transplanted into 4-inch pots. The plants were grown, inoculated, and incubated for the appearance of symptoms in the greenhouse.

The aphids were transferred from plant to plant with a camel's-hair brush. The plants, during the feeding periods of the vectors, were caged in the standard type of cage described by Severin *et al.* (6, 22). Following inoculation, the plants were usually fumigated with nicotine to kill the insects present.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Yellow-net of sugar beets has been noted in the field only in northern California. Leach has sent specimens from Sherman Island and Isleton, California. Specimens have been collected near Mt. Eden, California, and in the Rio Vista area. Thus far, diseased specimens found in the field have been limited in number and relatively isolated.

SYMPTOMS

The symptoms manifested in a typical specimen of the disease consist of a yellow network of veins and veinlets against a background of green

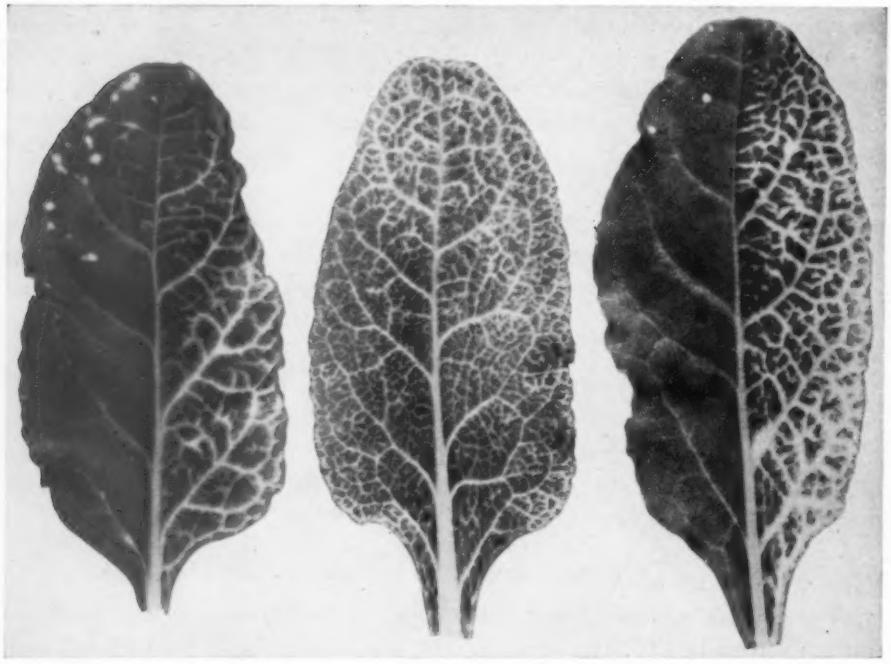

FIG. 2. Leaves from Swiss chard experimentally infected with the yellow-net virus showing variations in the magnitude and intensity of symptom expression.

interveinal tissue. The symptoms may appear within 9 days following inoculation of a small healthy beet seedling by means of an infective aphid. Usually the first symptoms are expressed on the youngest leaf of the plant. When the youngest leaf exhibits symptoms early in its development, the main lateral veins arising from the midrib often are the only ones affected by the chlorosis. As the leaf expands and matures, the chlorosis involves more and more of the veinlet network. It is from such symptoms that the name, yellow net, is derived.

Variation from the typical symptom pattern is of common occurrence, but it appears to be merely a difference in intensity of symptom development, both in the number of veins and veinlets affected and in the lateral dispersion of the chlorosis from the veinlets into the adjacent tissues.

Frequently one of the first recognizable symptoms found in an inoculated plant consists of one to several scattered yellow spots on a leaf blade (Fig. 2, left). The number, shape, and size of the spots vary, but the usual shape is circular and the usual diameter is approximately 1 mm. Occasionally, only one spot can be detected upon an entire plant.

Usually, following the appearance of the yellow spot phase, the yellow-net phase develops. The extent of involvement of the veinlet network varies, *i.e.*, in some cases only some of the larger secondary veins and larger veinlets are affected (Fig. 2, left), while in other instances it appears as if every vein and veinlet has been affected by the chlorosis (Fig. 2, center).

In some plants the chlorosis involves more area, by diffusing from the veinlets into adjacent mesophyll areas. This diffusion produces a pattern of wide yellow veins separated from one another by the green mesophyll tissue. The lateral diffusion may increase and the areas of spreading chlorosis coalesce, thus surrounding small areas of green tissue and producing a green island pattern. In severe cases this phase is followed by complete obliteration of the green islands of tissue by further diffusion of the chlorosis, and the leaf blade then is completely chlorotic. The color of the chlorosis may vary from cream to golden yellow under greenhouse conditions. The complete chlorosis is confined to the oldest leaves, while the younger leaves of such plants usually have a rather broad, diffuse form of the yellow-net symptom.

In older inoculated plants, the symptom expression may be confined to a yellow spot phase or a slight peripheral yellow net pattern. In some cases, as the plants grow older, the symptoms tend to become milder, or even disappear. Virus recovery trials from such plants have demonstrated that virus is still present, and that it will induce typical symptoms.

Other variations are those in which only certain portions of the leaf blade are affected. One of the more common examples of this type is an asymmetrical symptom expression by a leaf blade (Fig. 2, right). In other cases only the base of the leaf blade may be affected, while in others symptoms may be limited to one of the lower quarters of the blade. These variations probably are associated with the state of maturity of various portions of the leaf blade when invasion by the virus occurs.

The type of symptom expression and the apparent inability of the virus to be transmitted mechanically suggest that the virus is dependent upon the phloem for successful inoculation. Since the symptoms, at times, tend to fade in older plants, it would seem likely that the chlorosis is due in part to interference with plastid development, much as in the case of some other virus diseases (5).

APHID TRANSMISSION

The first diseased specimens were received in June, 1945. Initial juice transmission trials were negative. The diseased plants were potted and

when they were in a suitable condition, a colony of noninfective green peach aphids was introduced on to one of the diseased plants.

In the first trials, one apterous vivipara and four alate viviparae were transferred from the diseased plants to each of 5 healthy beets. The test feeding period was approximately 3 weeks, after which the plants were fumigated. One of the 5 inoculated plants manifested symptoms about 2 weeks later. The experiment was repeated, using groups of 25 apterae. and transferring them from the same naturally infected source plant to a second group of 5 healthy beet plants. Twenty-four days following the inoculation, one of the series exhibited symptoms of the disease. This preliminary work appeared to establish the virus nature of the disease, since the green peach aphid does not produce any severe toxic effect on the sugar beet in the course of its feeding. Moreover, the symptoms appeared in a systemic form following the removal, from the test plant, of an aphid population which previously had been fed upon a diseased plant.

MECHANICAL TRANSMISSION

Three methods of mechanical inoculation were used in attempting to transmit the yellow-net virus artificially: (1) the carborundum method (17), (2) the flamed needle technique after Severin (20), and (3) a multiple-needle method used in connection with work on the sugar-cane mosaic virus (18). Negative results were obtained in all of the trials.

INCUBATION PERIOD

The term, incubation period, in this paper, means that period of time elapsing between the inoculation of a healthy plant with the virus and the appearance of symptoms on that plant. Based upon 169 examples, taken at random from various experiments, the minimum incubation period required for the appearance of the first symptoms on young beet seedlings was 9 days, while the maximum was 49 days, with a mean of 24.42 days. The maximum undoubtedly is not a true maximum, but if an infection developed following this period of incubation, it was comparatively rare, or the chance for accidental infection might cast some doubt upon the validity of the recording.

AVAILABILITY THRESHOLD

The term, availability threshold, denotes the time, following inoculation, that intervenes until the virus in the host becomes available to a previously noninfective vector. The term is equal in meaning to the term minimum incubation period of Severin (19). The method used to determine the availability threshold was as follows. Healthy beet seedlings were infested with groups of 10 infective apterae. The following day the plants were fumigated and 7 days later a group of 10 noninfective apterae was placed upon each seedling. The 7-day period was allowed to elapse,

since preliminary trials had indicated that the virus was not available to a vector until approximately a week following inoculation.

The apterae were removed after a minimum infection feeding period of 15 hours and transferred to a healthy beet seedling. Following a test feeding period of 24 hours, the plants were fumigated. The procedure was repeated daily until symptoms appeared upon the originally inoculated beets, or until it was felt that a sufficient number of recovery trials had been made. Begining with the thirteenth day, the young which had been produced by the daily added groups of previously noninfective apterae were used to recover the virus. The results of the trials are given in table 1.

From the results obtained, the yellow-net virus becomes available to

TABLE 1.—*Results of trials to determine the availability threshold of the yellow-net virus, using groups of 10 previously non-infective green peach aphid apterae, an infection feeding period of at least 15 hours, and a test feeding period of 24 hours.*

Virus source beet no.	Incubation period in days	Day, following inoculation, that recovery trial was made [a]												
		7	8	9	10	11	12	13	14	15	16	17	18	19
1	12	—	+	—	—	+	—	+	....	....	....	....	....	....
2	12	—	—	+	—	—	+	+	....	....	....	....	....	....
3	20	—	—	—	—	—	—	—	—	+	+	+	+	+
4	14	—	+	—	—	—	+	+	....	....	....	....	....	....
5	23	—	—	+	+	+	—	+	+	+	+	+	+	+
6	17	—	—	—	—	—	—	+	—	+	+	—	+	....
7	17	—	—	—	+	+	—	+	+	+	....	....	....	....
8	17	—	+	—	+	+	—	+	+	+	+	+	+	+

[a] The plus sign (+) indicates the production of the disease, and the minus sign (—) shows that no disease resulted.

a previously noninfective insect, at a minimum of 8 days following inoculation. In relation to symptoms, the virus becomes available at a minimum of 3 days and a maximum of 14 days, with an average of 6.5 days, prior to the appearance of distinguishable symptoms.

DOUBLE VIRUS STUDIES

In order to test the hypothesis that there might be an interaction between other viruses commonly affecting sugar beets in California and the yellow-net virus, when two of them are present simultaneously in a single sugar beet plant, the following tests were made.

Five sugar beets, infected with the yellow-net virus and exhibiting good symptoms, were inoculated by the carborundum method with sugar-beet mosaic virus. Within 11 days following the treatment the symptoms of beet mosaic were evident upon the new leaves of all 5 plants, without the symptoms of yellow net. The plants were allowed to grow for approximately

1 month, at the end of which time a colony of noninfective green peach aphid was placed upon each plant. Following the completion of a 10-day feeding period, the aphids from each plant were transferred to a series of 5 healthy beet seedlings for 3 days. The beet mosaic virus was recovered only in the first series, and the yellow-net virus was recovered not only in the first series, but also in the series of each succeeding day. Table 2 gives the results in detail.

A second group of sugar beets, infected with the yellow-net virus and exhibiting good symptoms, was inoculated with the curly top virus by means of 5 infective male *Eutettix tenellus* (Baker). Four beets were inoculated, and following an incubation period of 3 to 4 weeks, all exhibited

TABLE 2.—*Results of trials to recover virus from sugar beets infected with both the yellow-net virus and the sugar-beet mosaic virus by means of previously noninfective green peach aphid apterae fed upon the infected beets* 10 *days and then transferred daily to lots of* 5 *healthy beet seedlings.*

Virus source beet no.	Virus recovered	Number of beets infected on three successive days			Total infected out of 15 inoculated
		1	2	3	
1	Mosaic	0	0	0	0
	Yellow net	2	3	5	10
2	Mosaic	0	0	0	0
	Yellow net	2	2	1	5
3	Mosaic	4	0	0	4
	Yellow net	3	3	1	7
4	Mosaic	0	0	0	0
	Yellow net	3	4	3	10
5	Mosaic	0	0	0	0
	Yellow net	4	5	3	12

symptoms of curly top on the newly developing leaves, again not accompanied by the symptoms of yellow net. After about 3 months, a noninfective colony of green peach aphid was introduced on to each of the 4 sugar beets, and 15 days later the aphids were transferred from each of the 4 beets to a series of 5 healthy beet seedlings. The yellow-net virus was recovered from each of the 4 sugar beets which was infected with the combination of viruses. Following the completion of this test, noninfective males and nymphs of the sugar-beet leafhopper were fed on the 4 infected beets, and 24 hours later were transferred from each of the 4 plants to 2 healthy beet seedlings. In this manner the curly top virus was recovered from each of the 4 beets.

In both instances of combination infection, the established virus, the yellow-net virus, was replaced in symptom expression upon the newly developing leaves by the introduced virus, either the sugar-beet mosaic virus or the curly top virus. This condition remained in effect for 3 to 4 months, after which time some of the plants exhibited symptoms of both viruses. In

any event, whether the plants exhibited a combination of symptoms, or only those of the secondarily introduced virus, the primary virus (yellow net) could be recovered by means of previously noninfective aphids, and recovered in a form which produced symptoms of typical pattern and severity in the recipient healthy beet seedlings.

Numerous workers (8, 12, 15, 25) frequently have used insects to separate combinations of viruses in plants.

From these tests, it is felt that all three of the sugar beet viruses used are distinct.

HOST RANGE

The following varieties of sugar and garden beets were tested and found susceptible to the yellow-net virus: U.S. 15, U.S. 22 (improved), U.S. 23, U.S. 33, G.W. 49, Detroit Dark Red, and Early Wonder. Swiss chard, *Beta vulgaris* L., var. *cicla* Hort., (Fig. 2) is susceptible to the virus, and manifests typical yellow net symptoms.

The following species of plants failed to develop symptoms when inoculated with the yellow-net virus.

Chenopodiaceae: *Atriplex patula* L., Spear orache; *A. rosea* L., Red orache; *Chenopodium murale* L., Nettle-leaf goose-foot; *Spinacia oleracea* L., Spinach.

Compositae: *Calendula officinalis* L., Pot marigold; *Callistephus chinensis* Nees, China aster; *Lactuca sativa* L., var. *romana* Hort., Romain lettuce; *Sonchus arvensis* L., Sow thistle.

Convolvulaceae: *Ipomoea purpurea* Roth, Morning glory.

Cruciferae: *Brassica campestris* (L.), Common yellow mustard; *B. chinensis* Baily, Pak-choi; *Capsella bursa-pastoris* (L.), Shepherd's purse.

Cucurbitaceae: *Cucumis sativus* L., Cucumber.

Geraniaceae: *Erodium moschantum* L'Her., White-stem filaree.

Plantaginaceae: *Plantago major* L., Common plantain.

Polygonaceae: *Polygonum coccineum* Muhl., Swamp knotweed; *P. persicaria* L., Lady's thumb; *Rumex crispus* L., Curly dock.

Solanaceae: *Datura stramonium* L., Jimson weed; *Lycopersicon esculentum* L., Tomato; *Nicotiana tabacum* L., Turkish tobacco.

SUMMARY

Yellow net, a previously unreported virus disease of the sugar beet, *Beta vulgaris* L., is described. The main symptom of the disease is a bright yellow chlorosis of the veins and veinlets of infected plants. The virus is not mechanically transmissible.

The green peach aphid, *Myzus persicae* (Sulzer) transmits the virus. The insect, after becoming infective, remains so for its life under experimental conditions.

The average incubation period of the virus in the sugar beet is 24 days,

with a minimum of 9 days. The virus may become available to a previously noninfective vector 8 days following inoculation.

Sugar beets can be infected simultaneously with yellow-net and beet-mosaic viruses, each in a recoverable state by insects, and the same is true of yellow net and curly top.

The host range of the virus appears to be limited, but all varieties of sugar beets and garden beets tested are susceptible to infection, as is Swiss chard.

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RHIZOCTONIA CAROTAE N. SP. AND GLIOCLADIUM AUREUM N. SP., TWO NEW ROOT PATHOGENS OF CARROTS IN COLD STORAGE[1]

WM. E. RADER

(Accepted for publication January 14, 1948)

Surveys of stored carrots made during 1943–1946 have shown some sixteen diseases to be present in cold and common storages in New York State. Two of these diseases were found to be caused by pathogens which are believed to be new. These new pathogens which occur in both mineral and muckland soils may cause damage during storage in some seasons amounting to 5 or 10 per cent of the crop from certain fields. They are described here for the first time under the names Rhizoctonia crater rot and Gliocladium rot.

RHIZOCTONIA CRATER ROT

Ramsey (7) reported in 1934 what is apparently the same disease as the one discussed here, although he gave no complete description of the organism. Specimens were shipped to him by one of the state inspectors at Rochester, New York. In 1942 the disease was brought to the attention of the staff at the Cornell Agricultural Experiment Station by the Fairport Storage Company. The disease apparently had been noted previously, but during that storage season losses were severe. During the next two years losses were light; however, again in the storage season of 1945–1946 the disease was found by the writer in epiphytotic form in several cold storages in New York State. At Albion, New York, by January 1, 1946 it was estimated that 10 per cent of the roots had lesions caused by this pathogen. In a cold storage at Fairport, New York, an average of 4 per cent of the roots were affected, with certain blocks running considerably higher. In one block of carrots in a cold storage at Williamson, New York, an estimated 5 per cent of the roots were affected.

Since the causal organism can flourish at temperatures below those maintained in cold storages, this disease may reach epiphytotic proportions and cause heavy losses in cold storages provided the relative humidities are high.

Symptomatology

Rhizoctonia crater rot has been found only on the roots which have

[1] Excerpt from a thesis presented August, 1946, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The author is indebted to Dr. A. G. Newhall, Department of Plant Pathology, Cornell University, under whose direction this work was conducted, for aid and suggestions in carrying out the research, to Mr. W. R. Fisher, Photographic Technician, for help in preparation of the photographic material and to Mrs. M. W. Allen, of the Botany Department of the same institution, for assistance in the preparation of the Latin diagnoses.

been kept in cold storage for two to three months. No visible symptoms are evident at harvest time. This disease is first evident when small, whitish hyphal knots of the pathogen appear on the roots (Fig. 1, A). Small pits

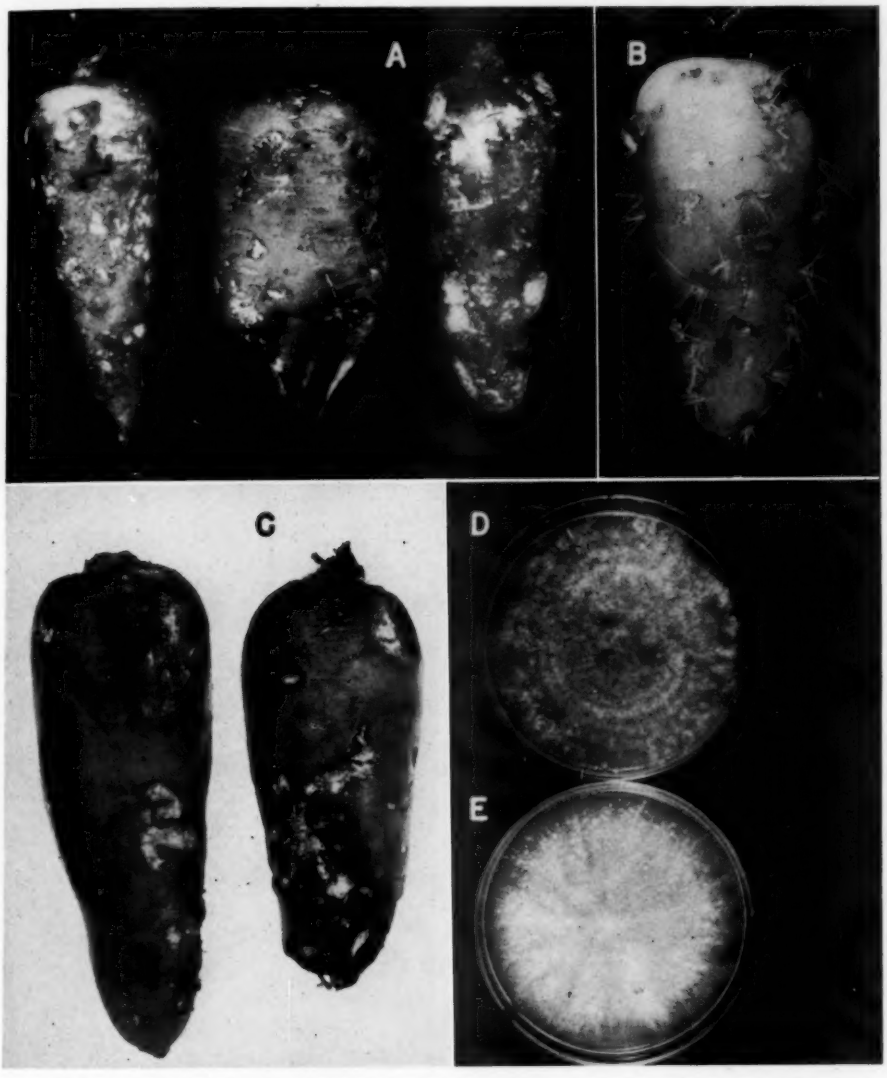

FIG. 1. Crater rot of carrots. (A) early stages of the disease on carrot roots, (B) cankers produced by artificial inoculation, (C) typical "craters" produced in the advanced stages of the disease, (D) culture of *Rhizoctonia carotae*, and (E) culture of *R. solani*, both incubated 18 days at 15° C. on potato-dextrose agar.

then appear under these hyphal knots. At this stage the disease can readily be confused with either the Fusarium dry rot, *Fusarium roseum* (Lk.) emend. Snyder and Hansen (*F. avenaceum* (Fr.) *Sacc.*), or the Gliocladium rot, *Gliocladium aureum*, described later. In many cases positive diag-

nosis of the cause of these small pits can only be made with the aid of a microscope. The characteristic mycelium of the pathogen can be readily identified. The pits enlarge into sunken craters lined with a white flocculent mycelium (Fig. 1, C). Because of these characteristic craters the common

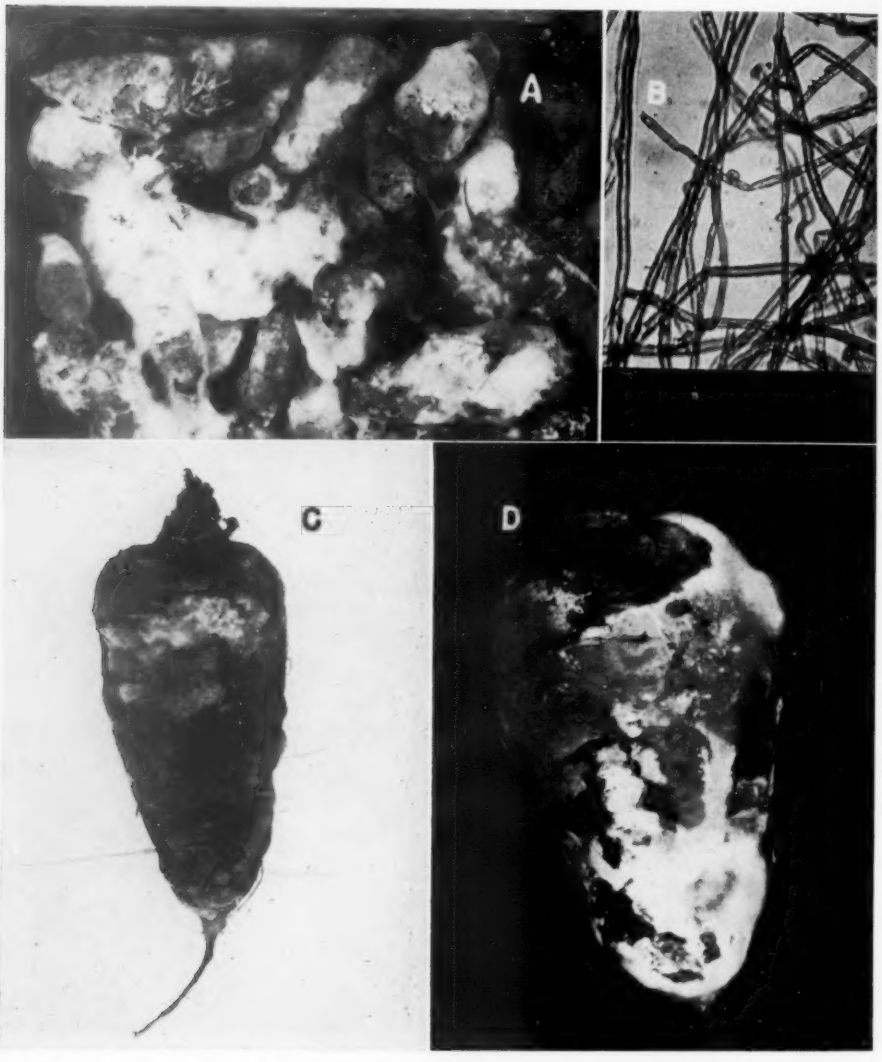

FIG. 2. Crater rot of carrots. (A) crate of carrots showing advanced stages of decay, (B) photomierograph of hyphae of *Rhizoctonia carotae* showing clamp connections, (C) crater rot lesion invaded secondarily by *Botryotinia Fuckeliana*, (D) crater rot lesion invaded secondarily by *Sclerotinia sclerotiorum*.

name "Rhizoctonia crater rot" is suggested for this new disease. The fungus spreads rapidly from these craters, in storages with high humidities, until entire crates are covered with the white cottony mycelium (Fig. 2, A). The disease at this stage has an appearance similar to the rot

caused by *Sclerotinia sclerotiorum* (Lib.) Debary, which often invades these diseased tissues secondarily (Fig. 2, D). It is distinguishable from the latter by the presence of a more loose, weftlike mycelium and by the absence of sclerotia. The decayed tissues beneath the surface lesions are light brown, usually firm and not watery.

Botryotinia Fuckeliana (Debary) Whetzel (*Botrytis cinerea* Pers.) and *Sclerotinia sclerotiorum* are found commonly as secondary invaders in these lesions (Fig. 2, C). The characteristic symptoms of Rhizoctonia crater rot soon become obliterated by these more rapid-growing fungi.

Pathogenicity

Carrot roots have been repeatedly inoculated with cultures from tissue plantings of diseased roots with the production of the typical firm rot associated with the disease in storages. The same organism has been recovered from these artificially inoculated tissues. The characteristic pitting, found commonly associated with the disease in storages, has been produced in the laboratory with difficulty. Apparently an external source of nutrients is necessary for the establishment of the fungus prior to penetration. Unwashed roots with some decaying organic matter present, or washed and surface disinfested roots inoculated with a hyphal suspension containing particles of agar and incubated at high humidities (at 4°-6° C.) usually had the typical pitting on the roots. The pathogen was always recovered from lesions produced on inoculated roots.

Cultural Characters and Morphology

The mycelium of this organism in culture or on carrots is white to slightly brownish, much lighter than the common strains of *Rhizoctonia solani* Kühn. (Fig. 1, D and E). The hyphae are 2.5 to 4.0μ. in diameter, branching, with a slight constriction immediately beyond the point of branching, septate, with the septa provided with 1 to 5 clamps (Fig. 2, B).

Sclerotia are readily produced in pure culture. Their cells are hyphalike in juvenile forms and barrel-shaped in more mature sclerotia. No spores or other fructifications have been found associated with this fungus either in culture or on infected carrots in storages.

In culture, isolates of this new pathogen were different in appearance than those of the common *Rhizoctonia solani* or *R. tuliparum* (Kleb.) Whetzel and Arth. The presence of white mycelium and golden yellow to light brown sclerotia with abundant clamp connections in the hyphae separate the crater rot pathogen from these other two common pathogens in this genus present in New York.

Matsumoto (6) has made use of the fact that fusion between hyphae of different isolates in culture indicates a close relationship of the fungi in identifying diverse strains of *Hypochnus saskii* Shirai. Numerous pairings of the carrot pathogen were made by the writer with several strains of *R.*

solani and *R. tuliparum*, but no fusions were found. Fusions were abundant in matings within strains of this new pathogen.

The presence of clamp connections in the mycelium of this fungus (Fig. 2, B) is indicative of the presence of the nuclear components necessary for sexual reproduction (1). Strains without clamp connections on the hyphae have never been isolated. Ramsey (7) recorded the presence of basidiospores in the lesions on the carrots which he examined, and designated the fungus as a *Hypochnus sp.* This presumably means that the spores were echinulate and colored. However, careful microscopic examination of many hundreds of diseased carrots failed to reveal the presence of any basidia or spores which could be assigned to this fungus. In the absence of any description of the organism by Ramsey, and the failure of the author to find any sexual spores, this organism is being placed in the form genus *Rhizoctonia* with the new name *Rhizoctonia carotae* being proposed and with the following description:

Rhizoctonia carotae sp. nov.

Hyphae hyaline to slightly brownish, 2.5 to 5.0 (mean 4.1) microns in diameter, richly branched, septate and the septa provided with 1 to 5 clamps. Mycelium white in mass on the surface of the suscept tissue or in culture. Sclerotia golden-yellow to brownish when old, irregular, 2.5 mm. in diameter, formed loosely in the hyphae; cells of the sclerotia barrel-shaped when old, hyphalike in juvenile forms, walls brownish to yellow, 4.1–7.1 (5.1) × 4.6–9.1 (6.2) microns, and germinating by a proliferation of one side of the cell. Pathogenic on carrot, producing a pitting and decay of stored roots.

Found commonly in cold storages in Orleans, Monroe, and Wayne counties, New York, on carrots which have been grown on both muckland and mineral soil.

Hyphae hyalinae vel aliquantum subfuscae colore, 2.5μ – 5.0μ (medio 4.1μ) diametro, copiose ramosae, septatae, septis 1–5 clamp-connexionibus ornatis. Mycelium album in massa in superficie tissues suscepti aut in cultura. Sclerotia aureo-flava vel subfusca quando antiqua, irregularia, 2.5 mm. diametro, laxe in hyphis formata; cellae sclerotiorum dolliformes quando antiquae, in statibus juvenilibus similes hyphorum, parietibus subfuscis vel flavis, 4.1–7.1 (5.1) × 4.6–9.0 (6.2)μ magnitudine, per prolificem in uno cellae parieto germinantesque. Pathogenica in Dauco Carota, foveas ruinamque in radicibus reservatis producens.

Vulgo inventa in promptuariis frigidis in comitatibus Orleans, Monroe, et Wayne Eboraci Novi in carotis qui vel in tellure stercuseo vel in metallico solo exculti sunt.

The type material and photographs have been deposited with the Department of Plant Pathology, Cornell University. Cultures also have been sent to the American Type Culture Collection, Washington D. C. and the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

Suscepts

Inoculations of this pathogen into celery, potato, beet, parsnip, and rutabaga did not result in infection. A white-colonied, clamp-forming organism was isolated from diseased parsnips that had been grown in

Monroe County, New York, where it was found causing a brownish rot similar to that produced by *Rhizoctonia solani*. This clamp-forming fungus appeared very similar in culture to *R. carotae;* however, inoculations into both carrots and parsnips did not produce infection. The known suscept range of *Rhizoctonia carotae*, at present, is limited to carrot roots.

Seasonal Development and Epiphytology of Rhizoctonia carotae

While little is definitely known regarding the life history of this pathogen, it is believed that the fungus may follow the same developmental pattern as some of the other species of *Rhizoctonia*. It is presumably a soil inhabitant. The disease is initiated prior to harvest time, or shortly after the roots are placed in storage. No visible symptoms are evident at harvest on carrots which later develop this disease. Carrots grown on land which was planted to other crops for several preceding years may subsequently show the disease in storage. The fact that the disease is not evident until after 1-2 months of cold storage does not necessarily indicate that infection takes place after the roots are stored, as incipient infections could take place in the field. The spotted appearance of the lesions on the roots is suggestive of penetration through areas left by dead secondary rootlets. Once lesions appear, however, the disease may develop fairly rapidly. A period of 2 to 3 weeks is usually required at 0° C. to completely destroy a root.

Storage containers may also serve as sources of primary inoculum for this disease. In one storage 19.5 per cent of over 600 isolations made from crates and baskets yielded this fungus. Direct microscopic examination has also repeatedly revealed its presence on storage containers.

The pathogen produces an abundance of mycelium over the affected areas. Adjacent roots are soon covered with the advancing mycelium and fall ready prey to its attack. The fungus grows along the wooden slats of the containers and spreads to other crates of carrots. In this way a series of secondary cycles may be initiated.

The difficulty experienced in demonstrating the several expressions of this disease in the laboratory is indicative of the rather narrow temperature and moisture requirements for infection and development of the pathogen. Observations in storages also support this contention. The greatest incidence of this disease was noted in two storages in which the humidities were maintained at near 100 per cent of saturation. The disease has never been found in common storages or in cold storages in which, by reason of construction or management, the relative humidity was held well below saturation.

The production of the pitting type of symptom in the laboratory has been produced only by wrapping inoculated carrots in wet paper toweling and placing them in a moist chamber at temperatures between 0° and 5° C.

(Fig. 1, B). The "well-method" of inoculation usually produces the typical rot associated with the pathogen.

Data presented in figure 3 indicate that on agar this organism grows over a temperature range of from –4° to 24° C., with the optimum being at 21° C. Infection has been obtained from 0° to 15° C. on carrots artificially inoculated and placed in moist chambers.

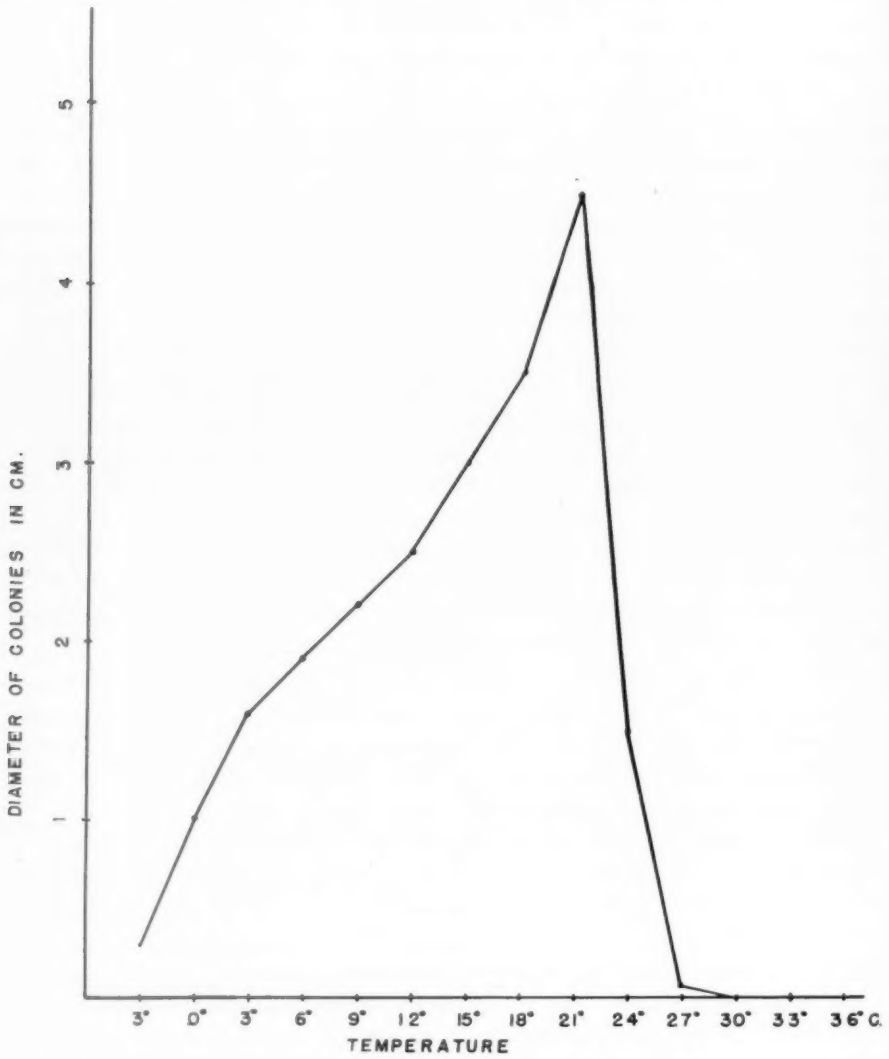

FIG. 3. Growth of *Rhizoctonia carotae* in Petri plates incubated for ten days at various temperature levels.

GLIOCLADIUM ROT

This disease occasionally caused only slight losses in stored carrots in cold storages in Monroe and Wayne Counties, New York. While losses

were never severe, the trouble was often encountered and usually confused with other diseases by storage operators.

Symptomatology

The lesions on carrots appear as shallow, slightly sunken cankers on the side of the root (Fig. 4, A). The decay is generally hard and leathery, rarely extending into the tissues more than a few millimeters. The affected tissues are light brown. The symptoms are very similar to those produced by *Fusarium roseum* (Lk.) emend. Snyder and Hansen (*F. avenaceum*

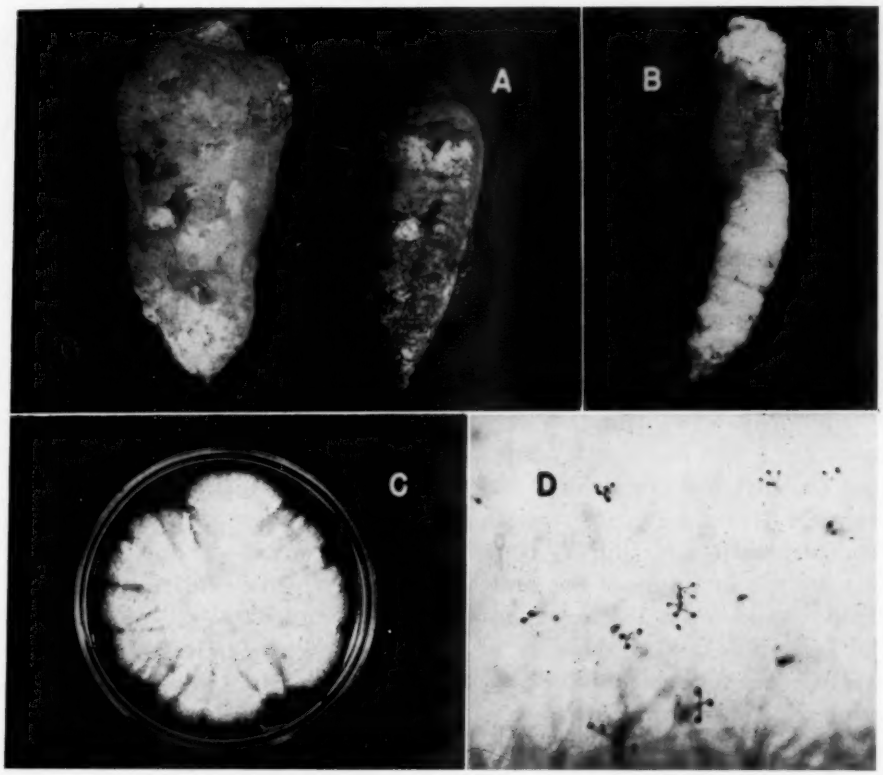

FIG. 4. Gliocladium rot of carrot. (A) shallow, hard lesions on roots from cold storage, (B) fructifications of pathogen on carrot root incubated 10 days at 21° C., (C) culture of *Gliocladium aureum* sp. nov. incubated 14 days on potato-dextrose agar at 21° C., (D) photomicrograph of Acrostalagmus type of conidial head found in young cultures of *G. aureum*. × 60.

(Fr.) Sacc.), a fungus with which the *Gliocladium* is frequently associated. Cankers caused by *F. roseum* which have dried out are practically indistinguishable macroscopically from those caused by the *Gliocladium*. The decay progresses slowly, turning the tissues brown and leaving the diseased area somewhat softer than the unaffected portion in roots inoculated by placing small bits of mycelium in holes bored in the carrots and incubated in moist chambers at 6° C. The affected area frequently is cov-

ered with a thick mat of mycelium (Fig. 4, B). The lesions are readily invaded by *Botryotinia Fuckeliana*, *Mucor hiemalis* Wehmer, or *F. roseum*, which quickly mask the characteristic lesions of this disease.

Etiology

Isolations from characteristic lesions have consistently yielded a fungus, which, by the characters of the conidia and conidiophores falls within the generic limits of the genus *Gliocladium*. Pathogenicity of this organism has been repeatedly demonstrated by artifical inoculations into carrots.

A careful study of the isolate from carrots has shown it to be very closely related to *Gliocladium roseum* (Lk.) Bainier. *G. roseum* was first described by Link (5) on dead stems of *Solanum* sp. under the name *Penicillium roseum* Lk. Lindau (4) listed this organism on dead stems of potato and *Hibiscus* sp., on decayed carrot roots, dead *Boletus* spp. and sclerotia in Germany, Belgium, Holland, and Ceylon. Saccardo (8) noted the fungus also as occurring on carrots in the Netherlands. Ravenel (Fungi Americani No. 571) collected this fungus (also under the name *Penicillium roseum*) from South Carolina on leaves of *Buxus sempervirens*. It has been noted by other authors as a laboratory contaminant and as occurring in the soil. As far as the writer has been able to determine *G. roseum* has never been found as a pathogen of living plants.

Sketches of an organism made by Shapovalov (9) under the name of *Clonostachys araucaria* DC. var. *rosea* Preuss. resemble very closely the pathogen found on carrots. The figures given by Shapovalov, however, are not *Clonostachys araucaria* in the sense of Lindau (4, page 345 and figure on page 347) since the sterigmata are not shown to branch in a verticillate manner, and the conidia are borne catenulately on the phialids. The conidia are not long-spicate nor are the spikes in a dense cluster as described by Saccardo (8, p. 165 in vol. 4) or Clements and Shear (2) and pictured by Lindau (4). The presence of a true penicillus is pictured by Shapovalov, some of the conidial clusters being united into a mucilaginous head typical of species of *Gliocladium*. Also, the Acrostalagmus type of head (10, figure 89; 3, page 442, figure 229, c) frequently found in cultures of *Gliocladium* spp. is also shown in Shapovalov's figure 2.

The distinct apiculum described by Shapovalov on the conidia of the organism that he found attacking potato has also been noted on the conidia of the carrot pathogen. The presence of the Acrostalagmus type of conidial head in conjunction with the true *Gliocladium* fructifications present in cultures of both the potato and carrot pathogens indicates that these organisms are probably the same species (Fig. 5).

Gliocladium roseum (*Penicillium roseum*) collected by Ravenel (Fungi Americani No. 571) and also by Meschutt (C.U.P.P. No. 6790)[2] on dead leaves of *Buxus sempervirens* has been examined and found to be different from the carrot pathogen.

[2] A specimen, No. 6790, determined by J. B. Ellis, filed in the herbarium of the Department of Plant Pathology, Cornell University, Ithaca, New York.

The general morphological characters of the pathogen found on carrot place it close to *Gliocladium roseum*. However, the slightly curved shape of the conidia combined with active pathogenicity, makes it seem advisable

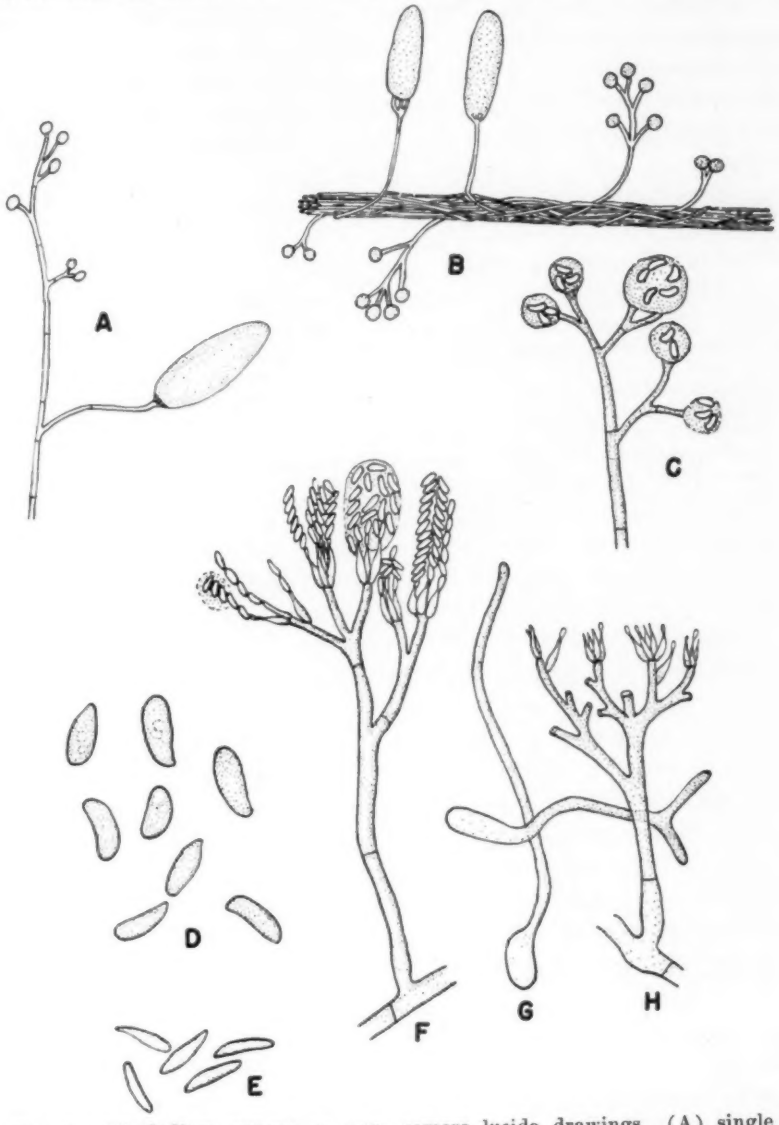

FIG. 5. *Gliocladium aureum* sp. nov., camera lucida drawings. (A) single hypha, bearing both Acrostalagmus and true Gliocladium types of fruiting structures, (B) hyphae bundled into strands (found commonly on agar cultures), (C) close-up of Acrostalagmus type of fruiting structures, (D) conidium of *G. aureum*, (E) conidia of *G. roseum* (*P. roseum*, Ravenel, Fungi Americani No. 571), (F and H) details of the penicillus of *G. aureum*, (G) germinating conidia of *G. aureum*, (A, B, approx. ×160; C, F, H, approx. ×480; D, E, G, approx. ×1450).

to distinguish this fungus from the common saprogen by designating it as a new species. The name *Gliocladium aureum* is proposed with the following description:

Gliocladium aureum sp. nov.

Colonies on potato-dextrose agar or Czapek's medium pure white at first, later pale yellow, occasionally light salmon to pink in the fruiting areas on Czapek's medium; mycelium loose-floccose, composed of simple hyphae or hyphae bundled in ropes; sclerotia not observed; conidiophores usually perpendicular to the medium, 40–120μ; penicillia up to 130μ in length, once or twice irregularly alternately branched; phialids 10–15 $\times$ 2–3μ. bearing conidia which may be aggregated in gelatinous masses or balls (Acrostalagmus type) or agglutinated in typical columns (resembling the Clonostachys type); conidia hyaline, elliptical, apiculate, smooth, 3.3 $\times$ 5.9μ. (extremes 2.6–3.6 $\times$ 5.1–6.7μ). A slight to marked yellowing of the medium is present in cultures on potato-dextrose agar.

Habitat: Pathogenic on stored carrot roots in New York.

Coloniae, in Solano tuberoso dextrose agari aut in Czapekis medio, primum purae albae, recentior pallidae flavae, pallidae salminae vel rosaceae in locis frugiferis in Czapekis medio; Mycelium laxe floccidum, compositum ex hyphis simplicibus aut ex hyphis in funes coactis; sclerotia non observata; conidiophoria plerumque ad medium perpendicularia, 40–120μ; penicillia ad 130μ longitudine, semel bisve verticillate aut irregulariter alterne ramosa; phialids 10–15 $\times$ 2.0–3.0μ ferentes conidia aut ad massas gelatinosas globulosve aggregata (Acrostalagmus typus) aut ad columnas typicas agglutinata (similes Clonostachys typi); conidia hyalina, elliptica, apiculata, laevia, 3.3 $\times$ 5.9μ (extremis 2.6–3.6 $\times$ 5.1–6.7μ) Levis vel notata medii flavido in culturis in Solano tuberoso dextrose agari adest.

Pathogenicum in Dauci Carota radicibus reservatis in Eboraco Novo.

The type material and photographs have been deposited with the Department of Plant Pathology, Cornell University. Cultures have also been sent to the American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

No data are available on the exact life history of this fungus. However, its similarity to species of *Penicillium*, both in distribution and cultural requirements would probably indicate a similar seasonal development. This would mean a succession of cycles on roots throughout the year whenever the suscept is present and proper environmental conditions exist.

Studies on the growth of this fungus at various temperatures indicate an optimum temperature between 21° and 24° C., a minimum below –3° C., and a maximum between 33° and 36° C. Infection has been obtained regularly on inoculated roots incubated in moist chambers at 6° and at 21° C.

DISCUSSION

The two diseases described are a part of a complex of four diseases causing pitting of stored carrot roots in New York. The other two diseases are caused by *Fusarium roseum* (Lk.) emend. Snyder and Hansen and by several species of *Typhula*. The *Typhula* occurs infrequently and the disease is characterized by the absence of hyphae in the lesions and by the typical brownish to blackish sclerotia on the affected areas. Frequently it is impossible to separate the diseases caused by *Rhizoctonia carotae*, *Gliocladium aureum*, and *Fusarium roseum* with certainty without microscopic examination. All three organisms produce pits varying in size from 2 to

3 mm. to several centimeters in diameter. These pits are usually lined with white hyphae. Typical expressions of these diseases permit separation as follows:

Rhizoctonia crater rot (*R. carotae*); sunken lesions lined with white mycelium, clamp connections present in the hyphae; affected tissues beneath the surface of the lesions light brown; in advanced stages hyphae readily spread to adjacent roots.

Fusarium rot (*F. roseum*); sunken lesions lined with white to yellowish tan or reddish mycelium, usually having a granular appearance due to the production of spores by the pathogen; hyphae lacking clamp connections, and rarely spreading to adjacent roots. Affected tissues are only slightly discolored, dry, and punky.

Gliocladium rot (*G. aureum*); lesions only slightly sunken, sparsely lined with light yellowish to pinkish mycelium; affected tissues light tan, very hard and leathery.

Gliocladium and Typhula rots are of less frequent occurrence and on the basis of present knowledge would not be expected to cause any serious losses in stored carrots.

Fusarium rot may become severe at temperatures of 15° to 20° C.; although its presence has been detected in carrots stored at temperatures between 6° and 35° C. While these temperatures occasionally exist in common storages this disease seldom causes any serious damage because of the low humidity in these storages.

The presence of Rhizoctonia crater rot in storage is dependent upon either field contamination or infection, or contamination from storage containers. Uninfected or uncontaminated roots placed in clean containers will not develop this disease.

Rhizoctonia carotae is the only pathogen in this group of pit producing fungi which causes any severe damage in cold storage. All experiments on control of this disease have given inconclusive results. Washing roots prior to storage has greatly increased the amount of soft rot (*Erwinia carotovora* (Jones) Holland) developing during the storage period with little effect on the incidence of Rhizoctonia crater rot. Dipping the roots in fungicides has not consistently controlled this disease.

Carrots stored in clean or disinfected crates and placed in storage rooms maintained at 0° C. and at a relative humidity of 90 - 95 per cent of saturation have shown a lower incidence of crater rot. This is the only control measure which can be recommended.

SUMMARY

Two diseases of carrots found in New York storages are described as new.

Rhizoctonia crater rot (*Rhizoctonia carotae* n. sp.) has been found caus-

ing severe losses in certain cold storages where the relative humidities have been high. This disease is characterized by sunken lesions lined with a white flocculent mycelium. These lesions may occur anywhere on the root. The affected tissues are light brown, usually firm and not watery. The hyphae in the lesions and in culture are hyaline to slightly brownish, 2.5 to 5.0μ (mean 4.1μ) in diameter, richly branched, 1–5 clamps at the septa. Sclerotia golden yellow to brownish, formed loosely on the hyphae, cells of the mature sclerotia barrel-shaped, and germinating by a proliferation of one side or the ends.

Gliocladium rot (*Gliocladium aureum* n. sp.) has been found in both cold and common storages in New York. This disease is characterized by shallow, slightly sunken lesions on the sides of the roots, which lesions are sparsely lined with light yellowish to pinkish mycelium; the affected tissues are very hard and leathery and light tan.

The only control believed recommendable is to store carrots at relative humidities below 95 per cent in new or disinfected containers.

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IMMUNITY OF CANADIAN BLACK CURRANT SELECTIONS FROM BLISTER RUST

GLENN GARDNER HAHN[1]

(Accepted for publication January 19, 1948)

The standard horticultural varieties of the European black currant, *Ribes nigrum* L., as commonly grown in this country are regarded as a definite menace to five-needle pines here because of their extreme susceptibility to white-pine blister rust, *Cronartium ribicola* A. Fisch. An investigation of these as well as a very large number of *Ribes* species for rust susceptibility, as listed by Spaulding (7), who reported his own work and that of his associates (7, pp. 17-24) a quarter of a century ago, did not reveal any black currant species resistant to blister rust. The early investigations, however, were lacking in studies of Asiatic black currant species from the probable original habitat of *C. ribicola* (3, p. 739) where rust-resistant ribes most likely would be found.

As the result of subsequent investigations of ribes susceptibility, the writer (2) found that varieties of *Ribes nigrum*, on the other hand, showed a marked resistance to piñon blister rust, *Cronartium occidentale* Hedgc., Beth., and Hunt. Accordingly, it was reasonable to expect that in the black currant group (1, pp. 29-38), as the writer proved subsequently for the red currant group (4), species and varieties would be found highly resistant to, or immune from *C. ribicola*. Experiments described in this paper are concerned with an Asiatic member of the black currant group.

CANADIAN EXPERIMENTS TO DISCOVER RUST-RESISTANT BLACK CURRANTS

In recent years a search was undertaken at the Central Experimental Farm, Ottawa, by Hunter and Davis (6) for suitable black currants for hybridization purposes in breeding for rust resistance. One black currant in particular from Siberia was used. It was called tentatively *Ribes nigrum*, No. 19/11. In 1939 Professor Alfred Rehder, Arnold Arboretum, Harvard University, identified it as *R. ussuriense* Janez., from eastern Manchuria, the twin species of *R. nigrum*, which, to quote Berger (1, p. 32) "differs [from *nigrum*] in many ways, and of quite a different odor." According to a communication received from Mr. D. S. Blair, September 23, 1941, concerning field tests carried on during 1935-40 at the Central Experimental Farm, the Asiatic black currant species, not tested for rust susceptibility in the United States, showed no signs of rust infection although it was cultivated in close association with *nigrum* varieties that became heavily infected with rust. *R. ussuriense*, according to Hunter and Davis (6), is apparently homozygous, rust-resistant.

Among the most desirable hybrids obtained were those that resulted from crossing pistillate *Ribes ussuriense* with staminate *R. nigrum* var. Kerry

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(6). The Kerry variety is of Canadian origin and is a seedling of Black Naples. Both of these standard *nigrum* varieties are rust-susceptible. Two selections from these crosses, namely No. 0-381 and 0-393 reported in this paper, represent separate clones respectively, each the vegetative progeny of a single seedling. Hunter and Davis (6) state that they show a marked resemblance in fruit characteristics to their cultivated parent, particularly with respect to vitamin C content and desirability for jam production. In the case of the Viking red currant (3), resistance to *Cronartium* is dominant to susceptibility; in the Canadian black currant selections (6), No. 0-381 and 0-393, the same inheritance appears to hold true.

INOCULATION RESULTS WITH CANADIAN BLACK CURRANTS

In the autumn of 1945, through the courtesy of Mr. M. B. Davis, Dominion Horticulturist, and Mr. A. W. S. Hunter, three plants each of the Canadian black currant clones No. 0-381 and 0-393 were received at New Haven for pathological testing[2]. The plants were grown first in pots in an unheated greenhouse. On April 11, 1946, the six plants were inoculated in the greenhouse with freshly collected aeciospores[3] from Connecticut, according to methods described previously (3, p. 741). Susceptible ribes "checks" were used to establish the viability of the inoculum and the suitability of the environmental conditions for infection. The checks included an undetermined variety of *Ribes nigrum*; the native black currant, *R. americanum* Mill.; and selections of a black currant propagated at the C. A. Hansen Nursery, Brookings, South Dakota, and reported as having been originally introduced from Siberia by Dr. N. E. Hansen in 1897 (5, p. 45; 8, p. 28). Through the courtesy of C. A. Hansen, the Hansen black currant selections were obtained in June, 1945. All of the checks became infected, the *nigrum* variety so heavily as to cause defoliation, whereas the other two black currant checks became only moderately infected. When inoculated, the plants of the two Canadian black currant clones had well developed leaves, which were in prime condition for the single test made that year. No signs of rust infection developed on them.

During the late summer of 1946 two plants of each clone were transplanted outdoors to a ribes plantation for testing under field conditions the following year. In 1947 the two plants retained in the greenhouse were

[2] Stock of the two clones, according to information received recently from Mr. Hunter, has also been distributed in the United States for horticultural testing to the following: Professor J. H. Clark, New Jersey Agricultural Experiment Station, New Brunswick, N. J.; Professor W. H. Alderman, Minnesota Agricultural Experiment Station, St. Paul, Minn.; Dr. W. E. Whitehouse, Division of Plant Exploration and Introduction, U. S. Department of Agriculture, Beltsville, Md.; Mr. G. L. Slate, New York Agricultural Experiment Station, Geneva, N. Y.; Professor R. E. Baker, University of California, Davis, Calif.; and Professor A. J. Riker, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

[3] The writer desires to express his appreciation to Messrs. J. E. Riley, Jr., Alton Miller, H. E. Yost, W. O. Frost, and H. G. Bradbury, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, for their cooperation in the collection of blister-rust aeciospore inocula used in the experiments reported in this paper.

inoculated on April 30 with fresh aeciospores of *Cronartium ribicola* collected in Virginia and later, on May 20, with the same type of inoculum collected in Connecticut. The susceptible ribes used as checks consisted of skunk currant, *R. glandulosum* Grauer, and a variety of *Ribes nigrum*. The two clones transplanted to the field were inoculated under conditions of humidity, temperature, and viability of inoculum that were highly favorable to infection. A test with inoculum from Connecticut was made on May 22 and a second test with inoculum from Maine on June 14. The susceptible field checks consisted of the same *nigrum* variety used in the greehouse and the Hansen black currant. Very heavy infection of *C. ribicola* occurred on the greenhouse checks; on the field checks infection was moderate on *nigrum* whereas that on the Hansen black currant was slight. There were no signs of rust infection on the Canadian black currants growing either in or outside the greenhouse[4]. Under the conditions of the tests the Canadian black currants appear to be truly immune from blister rust in the nice sense of that term, *i.e.*, completely free or exempt from rust. In this respect the black currant clones differ from the commercial Viking red currant, for at certain stages of leaf development in this particular ribes an invasion of germ tubes of *C. ribicola* occurs but the infectious agent is totally unable to establish itself and subsequently to produce its spores (4, p. 343).

RESULTS OF NATURAL EXPOSURE TO RUST AND MILDEW

During the tests of 1946 and 1947 uredospores of blister rust were present in the greenhouse and in the field, as indicated by natural infection of *Cronartium ribicola* throughout the season on leaves of susceptible ribes not inoculated artificially. Infection of the Canadian black currants was not observed to occur from inoculum of this type.

In 1946, after results of the rust-inoculation experiments on the Canadian clones in the greenhouse had been obtained, a natural infection of powdery mildew, *Sphaerotheca mors-uvae* (Schw.) Berk. and Curt., occurred on the new growth. Conditions in the unheated greenhouse were so favorable for mildewing that even the *nigrum* check, not ordinarily attacked, became affected. In the late spring of 1947 mildew again occurred in the greenhouse but it was not nearly so severe as in the previous year. It did not occur, however, on the Canadian clones in the field. These observations indicate, as Hunter and Davis (6) had previously observed, that the basis of resistance to blister rust in the two black currant clones is not operative with respect to mildew.

U. S. DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH
OSBORN BOTANICAL LABORATORY,
YALE UNIVERSITY, NEW HAVEN, CONN.

[4] In a letter dated October 4, 1947, Professor A. J. Riker informed the writer that the Canadian black currant clones sent him for testing have been growing in pots in a greenhouse along with other black currants. So far the former have not shown any blister rust whereas the latter have been severely rusted. Accordingly the Canadian clones are recorded to date as immune from rust inoculum collected in Connecticut, Maine, Virginia, and Wisconsin.

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EFFECT OF COPPER INJURY ON CONCORD GRAPES[1]

R. F. SUIT[2]

(Accepted for publication January 19, 1948)

INTRODUCTION

Ever since Bordeaux mixture was discovered and it was found that this fungicide would control downy mildew (*Plasmopara viticola* (Berk. and Curt.) Berl. and De Toni) on grapes (7), it has been generally considered that grapes were not particularly subject to copper spray injury. However, in 1941 a preliminary report (2) indicated that Concord grapes were subject to injury from sprays containing neutral copper fungicides at a copper content equal to that in a 4-4-100 Bordeaux mixture. The injury was characterized by reduced vine growth, smaller, yellowish-green leaves and reduced yield. Subsequently it was reported (3, 4) that the yield obtained the year following the spray application was reduced more, even though a copper fungicide was not applied. The second year following the injury, the yield from the injured vines had increased so that it was equal to that from vines which had not been injured (4).

Because of this apparently severe copper injury to Concord grapes, it was advisable to determine the exact effect of the injury over a period of years, the concentration of copper that would not produce injury and a method of alleviating the injury. Although Bordeaux mixture did not visibly injure the vines it could not be used with certain insecticides for insect control on grapes because of its alkaline reaction. Therefore, it was necessary that a neutral fungicide be used under some conditions.

MATERIALS AND METHODS

Through the cooperation of the Pomology Division at Geneva, New York, a block of Concord grapes, 80 years of age, was made available at the Vineyard Laboratory, Fredonia, New York for the investigation of copper injury. The block was of sufficient size so that 16 spray treatments could be used with duplicate plots of 30 vines each. There were no diseases present in this vineyard, but the grape berry moth (*Polychrosis viteana* Clem.) was present which made some modification of the spray schedule necessary (6).

The complete spray schedule was as follows:

Application I—When 3 or 4 leaves were present on the new shoots.

Application II—Before bloom.

[1] This investigation was completed while the author held the position of Assistant Professor, Division of Plant Pathology, New York State Agricultural Experiment Station, Geneva, New York. Approved by the Director for publication as Journal Paper No. 722, New York State Agricultural Experiment Station, Geneva, New York, October 15, 1947.

[2] The writer is indebted to K. W. Loucks, Associate Plant Pathologist, Florida Citrus Commission, stationed at the Citrus Experiment Station, Lake Alfred, Florida, for the statistical analysis of the data presented and is now Plant Pathologist, Citrus Experiment Station, Lake Alfred, Florida.

Application III—After bloom.
Application IV—Ten to fourteen days after application III.
Application V—When second brood grape berry moth began to appear.
Application VI—Ten to fourteen days after application V.

Thus a complete schedule including the control of the grape berry moth required 6 applications. Normally a maximum of 5 applications may be used for black rot (*Guignardia bidwellii* (Ell.) Viola and Ravaz.) control with the fifth application made 4 weeks after bloom.

Tribasic formulae

A. Tribasic copper sulfate 2–100
Rosin fish oil soap 1½–100

B. Tribasic copper sulfate 2–100
Black Leaf 155 (14 per cent) 3–100
Rosin fish oil soap ¾–100

C. Tribasic copper sulfate 2–100
Hydrated lime 4–100
Rosin fish oil soap 1½–100

D. Tribasic copper sulfate 2–100
Lead arsenate 3–100
Hydrated lime 4–100
Rosin fish oil soap 2–100
Kerosene—1 pint–100

E. Tribasic copper sulfate 1–100
Black Leaf 155 (14 per cent) 3–100
Rosin fish oil soap ¾–100

YCO formulae

A. Yellow cuprocide 1–100
Rosin fish oil soap 1½–100

B. Yellow cuprocide 1–100
S.E.C. oil[1] 1 pint–100

C. Yellow cuprocide 1–100
Black Leaf 155 (14 per cent) 3–100
Rosin fish oil soap ¾–100

D. Yellow cuprocide 1–100
Black Leaf 155 (14 per cent) 3–100
S.E.C. oil 1 pint–100

E. Yellow cuprocide 1–100
Hydrated lime 4–100
Rosin fish oil soap 1½–100

F. Yellow cuprocide 1–100
Hydrated lime 4–100
S.E.C. oil 1 pint–100

G. Yellow cuprocide 1–100
Lead arsenate 3–100
Hydrated lime 4–100
Rosin fish oil soap 2–100
Kerosene 1 pint–100

YCO formulae

H. Yellow cuprocide 1–100
Lead arsenate 3–100
Hydrated lime 4–100
S.E.C. oil 1 pint–100

I. Yellow cuprocide 10 oz.–100
Black Leaf 155 (14 per cent) 3–100
Rosin fish oil soap ¾–100

Lead and lime formula
Lead arsenate 3–100
Hydrated lime 4–100
Rosin fish oil soap 1½–100

Black Leaf formula
Black Leaf 155 (14 per cent) 3–100
Rosin fish oil soap ¾–100

Bordo formulae

A. Bordeaux mixture 2–2–100
Rosin fish oil soap 1½–100

B. Bordeaux mixture 2–2–100
Calcium arsenate 3–100
Rosin fish oil soap 2–100
Kerosene 1 pint–100

C. Bordeaux mixture 2–2–100
S.E.C. oil 1 pint–100

D. Bordeaux mixture 2–2–100
Calcium arsenate 3–100
S.E.C. oil 1 pint–100

E. Bordeaux mixture 4–4–100
Rosin fish oil soap 1½–100

F. Bordeaux mixture 4–4–100
Calcium arsenate 3–100
Rosin fish oil soap 2–100
Kerosene 1 pint–100

G. Bordeaux mixture 8–8–100
Rosin fish oil soap 1½–100

H. Bordeaux mixture 8–8–100
Calcium arsenate 3–100
Rosin fish oil soap 2–100
Kerosene 1 pint–100

[1] S.E.C. oil is self-emulsifying cottonseed oil supplied by Rohm and Haas Co.

Because of the variation in the insecticides used in the spray mixtures for the different applications (6) the number of spray mixture formulae necessary for the experiment was materially increased. In the 24 formulae listed, all materials are given as pounds per 100 gallons of water unless otherwise stated. In some of the spray mixtures it will be noted that 1 pint of kerosene is added per 100 gallons. This is added to reduce the foaming resulting from the use of excess rosin fish oil soap which was neces-

sary to give the mixture a suitable physical property so that it would wet and cover the grape berries with a satisfactory spray deposit (6).

The treatments were arranged to allow for plots sprayed with neutral copper fungicides alone, with different spreader stickers and with lime, while the Bordeaux mixture plots were sprayed with different concentrations and with different numbers of applications. The spray mixtures applied to the plots for each of the 16 treatments and for each of the 6 applications are given in table 1.

TABLE 1.—*Spray mixtures used for the various treatments*

Treatment No.	*Application*					
	I	II	III	IV	V	VI
1	Tribasic–A	Tribasic–A	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B
2	YCO-A	YCO-A	YCO-C	YCO-C	YCO-C	YCO-C
3	YCO-B	YCO-B	YCO-D	YCO-D	YCO-D	YCO-D
4	None	None	Lead & Lime	Lead & Lime	Black Leaf	Black leaf
5	Bordo-A	Bordo-A	Bordo-B	Bordo-B	Tribasic–E	Tribasic–E
6	Bordo-C	Bordo-C	Bordo-D	Bordo-D	Tribasic–E	Tribasic–E
7	Bordo-E	Bordo-E	Bordo-F	Bordo-F	Tribasic–E	Tribasic–E
8	Bordo-G	Bordo-G	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
9	None	Bordo-G	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
10	None	None	Bordo-H	Bordo-H	Tribasic–E	Tribasic–E
11	None	None	Black Leaf	Black Leaf	Black Leaf	Black leaf
12	Tribasic–C	Tribasic–C	Tribasic–D	Tribasic–D	Tribasic–E	Tribasic–E
13	YCO-E	YCO-E	YCO-G	YCO-G	YCO-I	YCO-I
14	YCO-F	YCO-F	YCO-H	YCO-H	YCO-I	YCO-I
15	Bordo-G	Bordo-G	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B
16	Bordo-A	Bordo-A	Tribasic–B	Tribasic–B	Tribasic–B	Tribasic–B

The spray mixture was applied at 350-lb. pressure with a trailing hose to which was attached a two-nozzle boom that had No. 4 discs in the nozzles. The amount of spray mixture used varied with the amount of growth on the vines. For application III the rate was about 225 gal. per acre, which is the amount recommended for satisfactory grape berry moth control and which would also give satisfactory disease control. In practically all cases the spraying of the plots for each application was completed in one day.

The experiment was carried on from 1941 to 1944 inclusive and the exact date on which each application was made during those years is given in table 2. In each year the crop of fruit was harvested and yield records obtained during the latter part of September.

RESULTS

The first indication of copper injury was a reduction in the amount of cane growth coupled with the yellowish-green color and reduced size of the leaves. Those rows in the vineyard which had copper injury could be noted from some distance because of the lighter green color. In 1941 and 1944 the early appearance of noticeable injury did not occur. However, in 1942, the injury was present on June 1, the date on which application

TABLE 2.—*Dates of spray applications*

Year	Application					
	I	II	III	IV	V	VI
1941	May 19	June 2	June 23	July 11	August 4	August 18
1942	May 19	June 1	June 23	July 7	July 29	August 7
1943	June 4	June 15	July 7	July 15	July 28	August 13
1944	May 25	June 6	June 26	July 11	July 31	August 11

II was made and prior to the blooming period. The injury was first noticed on July 7 in 1943, just after the blooming period and the date on which application III was made. In 1942 the injury appeared 13 days after the first application, while in 1943 it appeared 33 days after the first application. The vines had been severely injured by the cold during the winter of 1942-43 and were retarded in their growth.

A study of the effect of spray treatment on the amount of cane growth was started in 1942. Exceptionally heavy winds occurred during the latter part of May and broke off many of the canes that were being measured so that it was not possible to complete the experiment. In 1943, measurements were again made but, because of the injury from the cold during the previous winter, complete records could not be made. A summary of the measurements on some of the plots is given in table 3. The neutral coppers (treatments 1 and 2) and the Bordeaux mixture 8–8–100 (treatment 8) caused a greater reduction in cane growth than the other treatments and the growth was about half as much as that which occurred when copper was not applied (treatment 4). A complete series of measurements was made in 1944. As shown in table 4 there was no significant difference between treatments 1, 2, 5, 7, and 8 which comprised the neutral coppers and the three concentrations of Bordeaux mixture. Significantly more growth occurred in treatments 4, 11, 12, and 13 which were the two check plots and

TABLE 3.—*Effect of certain spray treatments on length of cane growth in* 1943.

Treatment No.	Principal fungicide	Inches of growth on				
		June 4	June 15	July 7	July 15	July 28
1	Tribasic	14.8 [a]	29.6	48.6	54.3	60.6
2	YCO	15.1	27.2	49.4	60.2	71.0
4	Lead and lime	16.3	38.9	89.5	117.8	144.4
7	Bordo 4-4-100	19.0	44.3	71.4	91.4	110.5
8	Bordo 8-8-100	14.6	28.8	52.0	63.2	75.6
13	YCO–Lime	18.8	37.2	69.8	84.8	98.6

[a] Average of measurement of from 5 to 10 canes in each plot.

the two neutral coppers plus lime. These differences occurred even though there was no apparent visible symptom of injury during the early part of the season. These results on cane growth indicate that the neutral coppers at a copper concentration equal to Bordeaux mixture 4–4–100 will cause severe reduction, that Bordeaux mixture usually causes some reduction, and that the neutral coppers with lime will cause the least reduction in growth.

Since the copper fungicides cause a reduction in the cane growth it is probable that a reduction in yield should also occur. The results from the yield records taken from 1941 to 1944 inclusive are given in table 5.

TABLE 4.—*Effect of certain spray treatments on length of cane growth in* 1944.

Treatment No.	Principal fungicide	Inches of growth[a] on						
		May 26	June 5	June 27	July 12	Aug. 1	Aug. 10	Oct. 4
1	Tribasic	13.5	28.8	42.7	52.3	60.8	64.6	72.5
2	YCO	13.5	28.6	43.1	55.7	65.2	72.7	82.0
4	Lead and lime	15.7	35.1	59.5	81.3	99.2	109.6	125.2
5	Bordo 2-2-100	14.2	29.4	46.9	55.8	65.7	69.4	75.9
7	Bordo 4-4-100	14.3	30.1	46.5	60.0	69.2	73.3	79.1
8	Bordo 8-8-100	14.5	29.1	46.9	61.8	72.1	78.9	86.7
11	Black Leaf	13.9	32.2	57.3	76.7	95.4	107.5	122.5
12	Tribasic–lime	15.6	31.6	53.2	75.1	91.3	99.1	112.3
13	YCO–lime	14.4	30.5	50.0	67.8	85.5	94.8	109.7
Least difference needed for significance—odds 19:1					18.28			
					odds 99:1 24.11			

[a] Average of measurements of 24 canes in each spray treatment.

There was no significant difference in the yield from any of the treatments in 1941. There was a significant reduction in the yield from treatments 1, 2, 3, 5, 6, 7, 8, 12, 13, 14, 15, and 16 in 1942. Although the yield in 1943 was reduced by the winter injury, there was a significant reduction in the yield from treatments 1, 2, 3, 7, 15, and 16. The 1944 results did not show as great a variation among the treatments and are somewhat of the same order as those obtained in 1941, although treatments 1, 13, and 16 gave a significant reduction in yield. In each of the four years, treatment 4 (lead and lime check) which did not contain copper gave the highest yields. Apparently the Black Leaf 155 (treatment 11) was somewhat injurious since the yields were slightly less than those from the lead and lime (treatment 4) although the difference was not significant. Considering the average

of the yields for the 4-year period, the data indicate that the neutral coppers (treatments 1, 2, 3) caused the greatest reduction in yield. Treatments 15 and 16 in which Bordeaux mixture was used for applications I and II and tribasic copper sulfate for applications III and IV, were equally injurious. Four of the Bordeaux treatments (No. 5, 6, 7, and 8) and one (No. 13) of the neutral copper resulted in a significant reduction

TABLE 5.—*Effect of spray treatments on yield*

Treatment No.	Principal fungicide	Yield[a]				4-year average
		1941	1942	1943	1944	
1	Tribasic	9.8	3.3	2.9	5.3	5.3
2	YCO	9.4	3.1	2.4	7.6	5.6
3	YCO	10.6	5.8	1.9	8.3	6.7
4	Lead and lime	11.3	14.8	6.9	10.0	10.7
5	Bordo 2–2–100	9.8	9.1	4.6	9.8	8.3
6	Bordo 2-2--100	9.3	10.5	4.7	8.8	8.4
7	Bordo 4–4–100	9.4	8.3	3.1	8.1	7.2
8	Bordo 8–8–100	9.1	9.3	4.3	8.4	7.8
9	Bordo 8–8–100	9.3	12.1	5.1	8.1	8.7
10	Bordo 8–8–100	9.2	12.7	4.8	8.0	8.7
11	Black Leaf	10.1	13.3	5.3	9.0	9.4
12	Tribasic–lime	10.6	10.6	4.9	8.5	8.7
13	YCO–Lime	9.4	10.5	4.6	6.7	7.8
14	YCO–Lime	10.1	10.6	6.1	7.7	8.6
15	Bordo–Tribasic	8.1	7.8	2.5	7.1	6.4
16	Bordo–Tribasic	9.1	7.7	2.1	6.9	6.5
Least difference needed for significance	Odds 19:1	3.06	3.06	3.06	3.06	2.17
	Odds 99:1	4.23	4.23	4.23	4.23	2.90

[a] Yield—average yield in pounds of fruit per vine from duplicate plots of 30 vines each.

in yield compared to that from the lead and lime (No. 4) check. The other treatments did not differ significantly in yield from that of the treatment 4.

While recording the yield data in 1942 it was observed that the bunches of grapes harvested from the vines in the plots which had copper injury appeared to be small. Therefore, when the yields were taken in 1943 and 1944, weights of 100 bunches from each plot were recorded (table 6.) The data show that those treatments which caused a significant reduction in yield (table 5) also caused a significant reduction in the size of the bunches.

The data presented show that copper injury on grapes causes a reduction in cane growth, yield, and size of bunches. However, this difference was not so apparent in some years as in others. It has been reported (1) that wet weather gives favorable atmospheric conditions for copper injury on apples. Copper injury was first observed on grapes in 1940 (2, 4) and caused a reduction in yield. In the experiments herein reported, the greatest reduction in yield occurred in 1942 and 1943. An examination of the weather records for May, June, July and August from 1940 to 1944,

TABLE 6.—*Effect of spray treatment on size of bunches*

Treatment No.	Principal fungicide	Weight (in pounds) of 100 bunches		
		1943	1944	2-year average
1	Tribasic	16.2	13.5	14.8
2	YCO	16.6	14.9	15.8
3	YCO	14.0	14.1	14.1
4	Lead and lime	20.0	18.1	19.0
5	Bordo 2–2–100	18.0	16.6	17.3
6	Bordo 2–2–100	17.8	15.7	16.8
7	Bordo 4–4–100	18.3	16.1	17.2
8	Bordo 8–8–100	18.2	15.3	16.8
9	Bordo 8–8–100	17.5	17.3	17.4
10	Bordo 8–8–100	18.2	17.6	17.9
11	Black Leaf	20.0	17.6	18.8
12	Tribasic–lime	20.5	16.8	18.6
13	YCO–Lime	20.7	16.5	18.6
14	YCO–Lime	21.7	16.9	19.3
15	Bordo–Tribasic	16.8	14.8	15.8
16	Bordo–Tribasic	17.0	15.6	16.3
Least difference needed for significance	Odds 19:1	3.5	3.5	2.5
	Odds 99:1	4.7	4.7	3.4

inclusive, gives some interesting information. There were 50 or more days with 0.01 inches of rain or more during these 4 months of the years when visible symptoms of copper injury appeared early in the season while there were only 38 and 35 such days in 1941 and 1944, respectively, when the early appearance of noticeable injury did not occur (Table 7). This indicates that wet weather is probably the principal factor influencing the occurrence of copper injury on grapes.

DISCUSSION

The following neutral copper fungicides have been found to cause injury to Concord grapes when used at a copper concentration equal to that in Bordeaux mixture 3–3–100: tribasic copper sulfate, copper compound A (4), Basicop (4), Yellow Cuprocide and Cuprocide 54-Y (4). Other neutral coppers were not tested. Bordow, which is a powdered Bordeaux mixture did not cause any significant injury (4). Throughout the various experiments on the control of grape diseases (5) after 1940, the neutral coppers were used at a copper concentration equal to Bordeaux mixture 2–2–100, or if used equal to Bordeaux mixture 4–4–100, there was 1 lb. of hydrated lime added to the spray mixture for each ¼ lb. of actual copper present in 100 gallons. In no case did apparent copper injury occur

TABLE 7.—*Records of rainy days*[a] *and rainfall*

Location (County)	Year	*May*		*June*		*July*		*August*		*Total*	
		Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall
		No.	*Inches*	*No.*	*Inches*	*No.*	*Inches*	*No.*	*Inches*	*No.*	*Inches*
Seneca	1940	17	5.35	13	4.37	13	3.13	11	2.52	54	15.37
Chautauqua	1941	12	1.68	9	2.61	10	4.04	7	3.16	38	11.49
Do	1942	19	5.57	11	1.48	15	5.59	12	8.88	57	21.52
Do	1943	20	4.06	7	2.64	10	2.48	13	3.49	50	12.67
Do	1944	11	2.76	10	3.47	7	0.82	7	2.36	35	9.41

[a] Rainy Days=Days with 0.01 inches of rain or more.

with either of these spray mixtures. If neutral coppers are to be used alone to give a non-alkaline spray mixture, there should not be more than ½ pound of actual copper per 100 gallons of spray mixture (5). As shown in the experiments reported herein, the copper injury can be practically eliminated by the addition of the hydrated lime to the neutral coppers when they are used at a higher copper concentration in the spray mixture. S. E. C. oil was used as a spreader-sticker in the spray mixture when copper injury was first observed. There was no difference in the amount of copper injury that occurred when either rosin oil soap or S. E. C. oil were used.

Contrary to what might be expected, there was no significant difference in the yield from vines sprayed with Bordeaux mixture 2–2–100, 4–4–100, or 8–8–100. All of the Bordeaux plots showed a significant reduction in the 4-year average yield compared to that of the check. If the application I or applications I and II were omitted when Bordeaux mixture 8–8–100 was used, there was no significant difference in yield as compared to the check. The yield was not significantly greater than when the applications were not omitted.

Since the visible copper injury symptoms in the neutral copper plots

usually appeared by the blooming period, it is indicated that most of the injury occurs when applications I and II are made. However, in two treatments Bordeaux mixture was used for applications I and II and tribasic copper sulfate at 2–100 for applications III and IV. The yields from these treatments were reduced in the same order as the yields from the treatments which received the neutral copper without lime throughout the season.

In general the neutral coppers gave significantly more injury than Bordeaux mixture. When hydrated lime was added to the neutral copper the injury was alleviated and there was no significant difference in yield between plots with this spray mixture and Bordeaux mixture or the non-sprayed plots. In order of decreasing injury, the spray treatments ranked as follows: neutral coppers, Bordeaux mixture, neutral coppers plus lime, and non-sprayed. The weather appeared to be a contributing factor as to the presence and severity of copper injury on Concord grapes.

The effect of the fungicides on yield was obtained in a vineyard where no diseases were present. If diseases had been present, the yield from the sprayed plots would have been greater than the yield from the non-sprayed plots.

SUMMARY

Following the observation in 1940 that neutral copper fungicides used at copper concentrations equal to that of Bordeaux mixture 4-4-100 would cause injury to Concord grapes, a four-year investigation was made to determine the factors involved.

Copper injury caused by neutral copper fungicides is characterized by small, yellowish leaves, reduced cane growth, and reduced yield. With Bordeaux mixture the reduction in size of the leaves and the change in color were not apparent.

Tribasic copper sulfate, copper compound A, Basicop, yellow cuprocide and cuprocide 54-Y were the neutral coppers used and all of them caused injury (5). In the 4-year experiment, tribasic copper sulfate and yellow cuprocide caused a significant reduction in cane growth, yield, and size of bunches.

The injury caused by the neutral coppers was alleviated if they were used at a concentration of not over one-half pound of metallic copper per 100 gallons, as previously reported (5), which would be a neutral spray mixture, or at higher concentrations if one pound of hydrated lime was added for each one-fourth pound of metallic copper per 100 gallons.

Bordeaux mixture caused a significant reduction in yield although visible symptoms of copper injury were not present. There was no significant difference between the yields from plots sprayed with 2-2-100, 4-4-100, or 8-8-100 concentrations of Bordeaux mixture.

The amount of copper injury that occurred was not influenced by either the rosin fish oil soap or the S.E.C. oil used as a spreader-sticker.

The spray treatments would rank in order of decreasing injury as follows: neutral coppers, Bordeaux mixture, and neutral coppers plus lime.

One factor contributing to the severity of the copper injury was the number of days with 0.01 inches of rain or more during May, June, July, and August.

There were no diseases present in the vineyard at any time during the course of the investigation.

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RESISTANCE TO SPOTTED WILT IN TOMATO

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Spotted-wilt disease of the tomato (*Lycopersicon esculentum* Mill.) has been observed by the writer occasionally in past years in New Jersey and neighboring states. It has not appeared to be a very common disease nor has a locally severe outbreak been recognized until recently. The reason for the infrequency of its occurrence has not been obvious, for elsewhere in the world, notably in Australia (5), in New Zealand (2), and in the Union of South Africa (4), spotted wilt has proved disastrous in its effects on tomato cultivation.

The disease known as ringspot of dahlia (*Dahlia pinnata* Cav.), studied originally by Brierley (1), has yielded a virus similar to that from spotted wilt of tomato in greenhouse and laboratory tests. Infected plants of dahlia are a probable source of much of the tomato disease. The causative virus can overwinter in dahlia corms whereas it usually cannot overwinter in tomato, a short-lived perennial species that is grown in the New Jersey area commonly as an annual crop and to a much less extent as a winter crop in greenhouses. Ringspot of dahlia, like spotted wilt of tomatoes, has been a relatively unimportant disease in the past in the eastern United States.

An unexpectedly severe outbreak of ringspot was observed in an extensive planting of dahlias near Vineland, New Jersey, in 1946. In this planting, slightly more than 30 per cent of the plants were obviously affected by October 10. Probably additional plants were infected but were not readily recognized as diseased at the time. Tomato plants had failed that year, and for a few years preceding had been difficult to grow, in the immediate neighborhood.

A study was made of the affected dahlia plots with a view to learning why ringspot disease, which is believed to be spotted wilt as it occurs in dahlia, should be so common there and what factors may have influenced an unprecedented spread to tomatoes. A third plant was found to be involved conspicuously. Chickweed (*Stellaria media* L.) was very abundant throughout the dahlia planting. It showed many white or yellow circular spots on old leaves and some distorted young leaves, such as had not been seen elsewhere. From affected chickweed leaves, a virus comparable to that isolated from ringspot lesions in dahlia was obtained by subinoculation to plants of *Nicotiana glutinosa* L. and tomato. Moreover the disease on chickweed could be reproduced readily by mechanical inoculation of healthy chickweed plants with virus originally derived from ringspot lesions in dahlia. Despite the potentially wide host range recognized for spotted-wilt virus (*Lethum australiense* H.), no other weed in the dahlia

fields appeared to be affected. There were present, however, abundant thrips, presumably including the ubiquitous onion thrips, *Thrips tabaci* Lind. (Thripidae), a vector of spotted wilt.

The severity of the local outbreak of the disease can be accounted for largely on the basis of extensive dahlia culture during the months of June to October, in an area heavily infested with chickweed, a winter annual occurring most abundantly from September to July. The seasons for growth of dahlia and chickweed overlap and together encompass the entire year. Transfer of virus from infected chickweed would tend to increase the incidence of spotted-wilt lesions in dahlia each summer; return of virus to seedling chickweeds in the autumn would complete a cycle. Chickweed alone could not be expected to maintain the disease, because the weed does not persist through the summer season in any quantity; and, in fact, fields a few miles removed from dahlia culture had only healthy chickweed plants. By March of 1947 the chickweed in the dahlia-growing area had been destroyed by intensive cultivation except in a small plot. The plants in this plot were so numerous as to touch each other over most of the surface of the ground. Almost all were diseased. Shortly afterward these also were destroyed with a view to attempting control of the disease. Had they been left to grow until July, much renewed spread of disease to dahlia might have been anticipated.

Varieties of tomato resistant to spotted wilt in the Territory of Hawaii had become available recently through the work of Kikuta, Hendrix, and Frazier (3), who developed the Pearl Harbor tomato and several improved derivatives of this variety. The writer had observed the adequacy of resistance of the Pearl Harbor tomato in Honolulu. It seemed possible that the outbreak of disease in New Jersey might be controlled, so far as tomatoes were concerned, by the use of such resistant lines. The Hawaiian investigators kindly supplied suitable seeds for this experiment.

Unfortunately, it was found that the New Jersey disease overcame the type of resistance that is characteristic of the Pearl Harbor tomato, both under conditions of natural spread in the field and under experimental conditions in the greenhouse. In the field a partial but insufficient resistance was displayed (See table 1). In the greenhouse, where infection was more nearly simultaneous and hence involved on the average younger plants and where dosage of virus was probably greater, hardly a trace of resistance was noted in the Pearl Harbor tomato and its derivatives. All inoculations in the greenhouse were made by mechanical means, that is, by rubbing leaflets in the presence of freshly expressed extracts of 10 to 12-day-diseased tomato plants and fine (320-mesh) carborundum powder.

Fortunately, seeds of a large number of unusual varieties of tomato were available in the writer's seed collection at the time; many were grown and seedlings produced from them were tested by mechanical inoculation. The virus used to test them was isolated originally from the dahlia by sub-

inoculation to *Nicotiana glutinosa* and subsequent transfer from the characteristic necrotic-type lesions on this plant to seedlings of Bonny Best tomato, a fully susceptible variety. In tomato, it produced tip blight symptoms and other manifestations characteristic of the tomato malady in the field. In expressed juice this virus was completely inactivated in 10 minutes at 45° C. To maintain the virus at sufficiently high titer for use in testing seedlings, subinoculations were made at approximately 10-day intervals to new young tomato plants. Tests of the virus when thus regularly transferred showed sufficiently high infectivity to produce, in one test, an average of 42.8 lesions per leaf in 10 leaves of *N. glutinosa*.

Most of the varieties tested proved highly susceptible but two outstandingly resistant lines of tomato were found.

One of the resistant tomatoes was known as Rey de los Tempranos (King of the Earlies). It was an Argentinian variety originally obtained

TABLE 1.—*Comparison of tomato varieties in the field at Vineland, N. J., on July 15, 1947*

Variety	Number of plants			
	Dead	Severely diseased	Moderately affected	Apparently healthy
Rutgers	26	10	4[a]	6[a]
Rey de los Tempranos	0	0	7	83
Pearl Harbor	17	40	6	27

[a] Only plants in the last two columns, moderately affected and apparently healthy, had produced appreciable amounts of fruit.

through the kindness of Dr. M. F. Babb, of the Cheyenne Horticultural Field Station in Cheyenne, Wyoming. All plants of this variety seemed to be resistant (See figure 1). Many of them showed no obvious response to inoculation, though a little virus could be recovered sometimes from inoculated leaflets, indicating localized and inapparent infection. A few became diseased systemically, but later sent out healthy branches and produced fruits. Seeds from a fruit on such an injured plant were grown. A progeny of 56 plants produced from them seemed as resistant as the variety itself, only two being moderately affected, whereas all of 44 plants of the susceptible variety Bounty, grown as controls, became systemically diseased. In the field, resistance proved to be of a similarly high order; a few plants became affected but the planting as a whole appeared vigorous and fruitful, whereas neighboring plantings of the varieties Rutgers and Pan America were so severely affected as to produce no usable fruits.

The second variety of value was the Manzana (Apple) tomato. This was another Argentinian variety, received through the kindness of Dr. F.

Rosenbusch of Buenos Aires. In this stock, the seeds of which were 10 years old when tested, some inhomogeneity existed. Part of the plants proved susceptible, but progenies that seemed wholly resistant were grown from seeds of resistant individuals.

First-generation hybrids between the susceptible Rutgers tomato and the resistant Rey de los Tempranos were not so resistant as the variety Rey de los Tempranos nor so readily infected systemically as plants of the seed parent, Rutgers. The same was true of a similar cross having Bounty as susceptible parent, instead of Rutgers, and of the reciprocal hybrid between Rey de los Tempranos, used as female parent, and Bonny Best, a

FIG. 1. An unaffected tomato plant of the resistant variety Rey de los Tempranos and a severely injured plant of the susceptible variety Rutgers, each 17 days after inoculation with spotted-wilt virus originally isolated from ringspot of dahlia in New Jersey. (Photograph by J. A. Carlile.)

fully susceptible sort, used as male parent. In all cases the F_1 plants seemed intermediate between the parental types in their responses to inoculation.

In a second generation of the Rutgers cross, grown from seed produced by self-pollination of flowers on the F_1 plants above mentioned, typical spotted-wilt disease was established in 299 plants of 401 inoculated; the remaining 102 plants reacted like the resistant variety Rey de los Tempranos, only 18 becoming somewhat affected later and these tending to send out healthy branches and to produce fruits. All of 397 inoculated control plants of the susceptible variety Rutgers became systematically diseased. The ratio of typically diseased to resistant plants among the second genera-

tion hybrids, 299:102, suggested that segregation for resistance had occurred in approximately a 3:1 ratio. A single-gene difference thus seemed to account for much if not all of the essential distinction between the resistant and susceptible stocks used in this test. The gene for resistance may be treated as though it were fully recessive in breeding experiments, although F_1 heterozygotes were shown to be more difficult to infect systemically than were the susceptible parent plants.

The Manzana tomato, as represented by selected resistant lines, was found to be as resistant as the variety Rey de los Tempranos in subsequent greenhouse and field tests in New Jersey. Manzana fruits were nearly spherical and of large size; they were red in color and of good flavor. Their only obvious defect was a tendency to concentric cracking and somewhat delayed ripening near the stem end of each fruit. Fruits of the variety Rey de los Tempranos were far more numerous and earlier in ripening, but were relatively small, flattened, and ridged.

The high degree of resistance of the variety Rey de los Tempranos in the field, the extreme susceptibility of the Rutgers tomato, and the intermediate but insufficient resistance of the Pearl Harbor tomato under New Jersey conditions are well shown in table 1.

Through the kindness of Mr. K. Kikuta and Dr. W. A. Frazier of the Agricultural Experiment Station at the University of Hawaii in Honolulu, Territory of Hawaii, the varieties found resistant in the field under New Jersey conditions were exposed to natural infection in Honolulu, on the grounds of the Agricultural Experiment Station, where a special test plot has been maintained for a study of resistance to Hawaiian spotted-wilt virus. Both Rey de los Tempranos and Manzana tomatoes appeared somewhat less resistant to spotted wilt there than the locally adapted resistant variety Pearl Harbor but much more resistant than the susceptible varieties Rutgers and Bounty. If the results of the preliminary test should be confirmed, this might indicate that the two types of resistance could be combined to advantage by crossing Pearl Harbor with Rey de los Tempranos or Manzana tomatoes. Among segregating progenies, some lines capable of resisting an increased variety of strains of spotted wilt might be found.

DISCUSSION

Spotted wilt is a viral disease of world-wide distribution. It has reached every continent and many oceanic islands. Infection seems to be much more frequent in some areas than in others, probably in accordance with availability of suitable plant hosts to act as reservoirs and thrips to act as vectors of the causative virus. Measures for control of the disease have sometimes been based on isolation of crops from cultivated or weed hosts and sometimes on destruction of insect vectors. Until recently no success has been reported in the search for immune or highly resistant varieties.

The first adequate resistance in tomato to damage by locally-occurring strains of the virus was reported in 1945 by the Hawaiian group of investigators, Kikuta, Hendrix, and Frazier (3). These investigators introduced the Pearl Harbor tomato and several excellent derivatives, which are capable of withstanding the disease as it occurs in the Hawaiian Islands. The existence elsewhere of one or more additional strains of virus requiring another type of resistance in tomato is illustrated in the present investigation. Perhaps the virus will continue to disclose potentially dangerous strains, for which the now known types of resistance may be inadequate. Further selection of types of resistance may be needed to complete eventually the solution of the world-wide problem of control of spotted wilt in tomato.

Comparison of experiences in the Hawaiian Islands with subsequent studies in New Jersey teaches a somewhat unexpected lesson. Investigators in the Hawaiian Islands might logically have concluded that reintroduction of spotted-wilt virus would be without effect, because the Islands long have been infested by strains of spotted-wilt virus and in recent years have possessed also an adequate resistance in tomatoes. If spotted-wilt virus from New Jersey had been introduced, however, the adequacy of existing resistance in tomatoes would have been severely tested and perhaps nullified, unless the damaging new strains had found conditions there unsuited to their multiplication or transmission. Fortunately, adequate resistance for New Jersey strains of virus is now available if needed. The insight provided by tests of the Pearl Harbor type of resistance in New Jersey would suggest, however, that is not advisable to introduce a virus into a country, especially one normally protected by ocean barriers, even though strains of the virus are reported as present already.

In general it has been supposed that a virus strain is capable of giving rise to all possible derivatives wherever it exists. There may, however, be practical limitations to selection and establishment of some variants. If mutation of one strain gives rise to a derivative especially well suited to potential host plants in an environment, the derived strain may persist and even become dominant. This evolutionary process may go on through a series of steps. Conceivably it may also reverse itself, so far as mutation is concerned, but suitable hosts to support the whole chain of events required for a gross change in characteristics or for a stepwise return may not be available, especially in a limited environment, such as that of an island or a small group of islands. For this reason certain strains may not be developed, selected, and established locally.

It will be of interest to test the two now known types of resistance in all the areas of the world from which spotted-wilt disease has been reported. Will the first-described virus of spotted-wilt, that reported from Australia by Samuel, Bald, and Pittman (5), resemble that of the Hawaiian Islands, that of New Jersey, or neither of these, in its effect on tomato plants bearing the Pearl Harbor type and on those bearing the newly described type of

resistance? Will the so-called kromnek disease in the Union of South Africa (4) resemble spotted-wilt disease as first recognized in Australia in its relationship to the two types of resistance? Whereas it would be obviously unsafe to import spotted-wilt viruses from different continents for comparison with one another, it would seem to be a safe and expedient process to send seeds of resistant tomatoes to all affected areas.

SUMMARY

An outbreak of spotted-wilt disease in a small area of southern New Jersey was caused by a strain or strains of the causative virus capable of overcoming the resistance of the Pearl Harbor tomato and its recent derivatives as developed in the Hawaiian Islands. A heritable resistance adequate for control of the New Jersey disease was found in two kinds of South American tomato, the variety Rey de los Tempranos and some lines of the variety Manzana, both from Argentina. The new type of resistance, as exemplified in the variety Rey de los Tempranos, was inherited as a single Mendelian factor in crosses with the susceptible Rutgers variety of tomato; approximately one quarter of all plants (102 of 401) in the second hybrid generation displayed resistance comparable to that of the resistant parent.

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EFFECTIVENESS OF FUNGICIDES IN CONTROLLING BLACK ROT OF SWEET POTATOES

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In recent years black rot of sweet potatoes caused by *Ceratostomella fimbriata* (E. and H.) Ell. has been assuming greater importance in the commercial sweet potato areas in Louisiana. This situation is attributed to a general relaxation in the use of sanitary practices necessary for the control of the disease and to the introduction of the practice of washing potatoes immediately before packing and shipping. Black rot has caused some losses in stored, unwashed potatoes, but the greatest losses have occurred during transit to market in the early crop which is harvested and shipped in July and August. Affected potatoes as they reach the market are illustrated in figure 1, A.

A fungicide which would be effective against black rot and could be used without harmful effects on the freshly dug sweet potatoes after washing, would be of considerable value in preventing losses from this disease. Some attempts have been made to find such a fungicide. Daines[2] and Parris[3] have reported on the use of borax in the control of soft rot of sweet potato and Jeffers[4] has reported on tests of several fungicides for preventing infection of sweet potato slices by *Ceratostomella fimbriata*.

During the past five years a number of fungicides have been tested in Louisiana for their effectiveness in controlling black rot. The results of these tests are reported in this paper.

MATERIALS AND METHODS

The sweet potato variety Porto Rico was used throughout and all tests with the fungicides were made in essentially the same way. After the sweet potatoes were washed they were dipped in a heavy spore suspension of *Ceratostomella fimbriata* and then immersed for about 30 seconds in a water solution or suspension of the fungicide to be tested. The potatoes were then placed in large paper-lined crocks or crates. To serve as checks potatoes were dipped in the spore suspension, placed in crocks or crates and stored under the same conditions as the treated potatoes. After a period of approximately 15 days the potatoes were examined. Counts were made of the lesions on each potato and the average number of lesions per potato in each treatment was calculated.

[1] Credit is due Joseph E. Seagram and Sons Inc., Louisville, Kentucky, for support of a fellowship in connection with these studies.

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TABLE 1.—*Results of tests showing the average number of black-rot lesions per potato which developed on the treated and untreated lots*

Treatment		No. of tests	No. of potatoes used		Ave. no. of lesions per potato	
Fungicide[a]	Concentration[b] (Per cent) in water		Treated	Untreated	Treated	Untreated
Borax	2.50	1	22	22	0.5	8.0
	1.25	7	124	127	0.9	8.4
	1.00	1	19	24	0.9	5.4
	0.67	1	24	24	0.8	5.4
	0.63	5	79	79	1.2	10.4
	0.50	1	21	24	1.3	5.4
	0.31	2	21	20	4.8	11.0
Dithane D–14	1.00	3	75	73	0.4	5.8
	0.67	4	106	105	0.6	6.6
	0.50	3	51	51	0.9	5.3
	0.25	3	36	37	4.2	9.1
	0.13	1	10	10	11.5	19.5
Acetic Acid	1.00	1	10	10	4.9	13.4
Calcium hypochlorite	Saturated	1	11	10	12.5	13.4
Dithane Z–78	0.20	1	41	39	1.0	6.8
	0.10	1	39	39	1.5	6.8
Dowicide A	0.40	1	18	17	12.1	13.1
Dowicide B	0.40	1	16	17	4.4	13.1
Elgetol	0.67	1	9	10	5.2	13.4
8-Hydroxyquinoline sulfate	1.00	1	15	17	5.0	13.1
Isothan Q–15	0.50	1	16	17	10.7	13.1
Mercuric chloride	0.10	2	53	53	0.5	7.1
Phygon	1.50	1	40	39	1.4	6.8
	0.30	1	40	39	2.7	6.8
	0.18	4	42	40	2.6	9.1
	0.09	2	20	20	2.5	11.0
	0.05	1	10	10	5.7	19.5
Potat-O-Dip	0.60	2	53	53	5.5	7.1
Puratized N5E	0.50	3	37	37	1.0	10.3
Puratized 177	0.25	1	10	10	8.6	13.4
Puratized N5D	0.20	1	22	22	0.1	8.0
Thiourea	1.50	3	31	30	1.2	7.6
	0.75	2	20	20	4.7	11.0
	0.38	2	20	20	5.3	11.0
	0.19	2	19	20	5.5	11.0

[a] The borax used was 20 Mule Team Brand made by Pacific Coast Borax Company; Dithane D–14 is a liquid containing 25 per cent disodium ethylene bisdithiocarbamate; Dithane Z–78 is zinc dimethyl dithiocarbamate; Dowicide A is sodium *o*-phenyl phenate; Dowicide B is the sodium salt of 2,4,5-trichloro phenol; Elgetol is sodium dinitro-ortho-cresol; Isothan Q–15 is lauryl isoquinolinium bromide; Phygon is 2,3-dichloro-1,4-naphthoquinone; Potat-O-Dip is a powder containing not less than 31.0 per cent aromatic phenylphenols and 0.1 per cent aromatic hydroxynitrocresols; Puratized N5D (10 per cent active agent) and Puratized N5E (5 per cent active agent) are phenylmercuritriethanol ammonium lactate; Puratized 177 (20 per cent active ingredient) is para amino phenyl cadmium dilactate.

[b] Concentration was based on the commercial product or experimental sample used and not on active ingredients.

TESTS WITH FUNGICIDES

Seventeen compounds were tested and the results with these are summarized in table 1. Since the tests were carried out over a long period of time and under different conditions, the results with each compound are given in comparison with the checks for the tests in which the material was used.

Based on the average number of lesions which developed on the treated compared to the untreated sweet potatoes, the following compounds are considered as having given satisfactory control of black rot: (1) borax at 0.63 per cent or stronger, (2) Dithane D–14 at 0.5 per cent or stronger, (3) Dithane Z–78 at 0.2 per cent, (4) mercuric chloride at 0.1 per cent, (5) Phygon at 1.5 per cent, (6) Puratized N5E at 0.5 per cent, (7) Puratized N5D at 0.2 per cent and (8) thiourea at 1.5 per cent. Since mercuric chloride and the two Puratized compounds contain mercury, they should not be used for treating potatoes that are for human consumption until further analyses are made; but they are safe for use in surface-treating roots before

TABLE 2.—*The average number of black-rot lesions developed on inoculated sweet potatoes and on similar potatoes treated in different concentrations of borax*

Treatment	No. of potatoes used	Ave. no. of lesions per potato
Untreated—checks	39	6.8
Borax, 1.0 per cent	38	0.1
0.7	40	0.1
0.5	40	0.1
0.3	40	0.4
0.1	39	0.5

bedding them for slip production. The Dithane Z–78, Phygon, and thiourea treatments were somewhat less effective than the borax and Dithane D–14 treatments. Thus, borax and Dithane–14 seem to have the best possibilities for use in treating sweet potatoes for the prevention of black rot after the commercial washing process, provided the solutions are allowed to dry on the surface. Preliminary tests indicate that the borax treatments are not effective if the potatoes are washed in water after treatment. Borax-treated and untreated potatoes are shown in figure 1, B and C.

EFFECT OF WEAK SOLUTIONS OF BORAX

To determine the lower limits of concentration of borax effective in preventing the development of black rot on inoculated sweet potatoes, a test was made with different concentrations from 0.1 per cent to 1.0 per cent. The results of this test are summarized in table 2. Concentrations as low as 0.1 per cent borax gave striking reductions in the average number of black-rot lesions which developed per potato.

TESTS WITH MARKET SWEET POTATOES

In a test under commercial conditions approximately 2400 potatoes showing no evidence of black rot were obtained from a lot of potatoes which

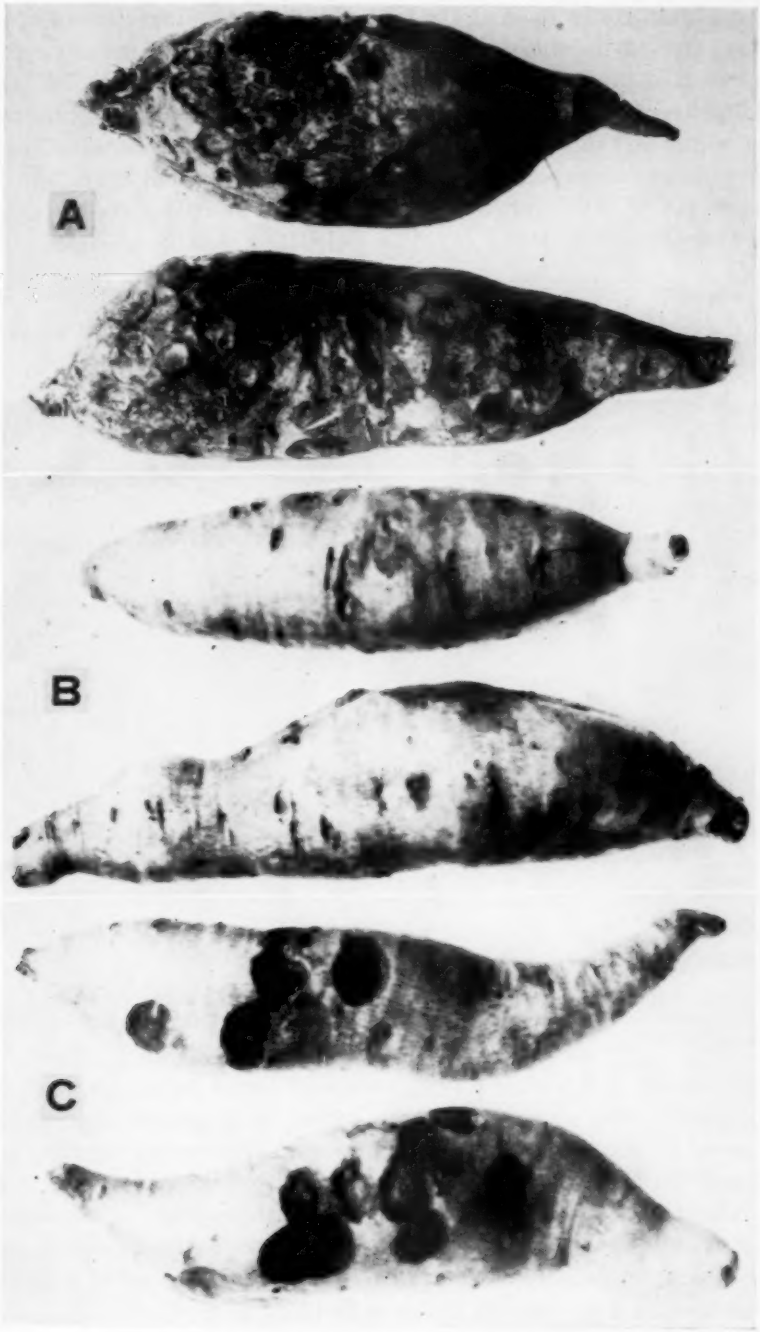

FIG. 1. A. Sweet potatoes which have developed black rot during transit to market. B. Sweet potatoes treated in a one per cent solution of borax after inoculation with *Ceratostomella fimbriata*. C. Sweet potatoes not treated after inoculation.

had some black rot in them at the time of washing. Immediately after the potatoes came off the washing machine, they were divided into four groups, one for each of the following treatments: (a) untreated checks, (b) dipped in a 1.3 per cent solution of Dithane D–14, (c) dipped in a 1.0 per cent solution of borax, and (d) dipped in a 0.6 per cent solution of borax. The potatoes were stored in paper-lined crates as for shipment and notes were taken two weeks later. The average number of lesions which developed per potato in the four treatments were: (a) 0.25, (b) 0.03, (c) 0.03, and (d) 0.05.

INJURY TO THE SWEET POTATOES RESULTING FROM TREATMENTS

In the borax treatments some chemical burning was observed on potatoes treated in 2.5 per cent solutions (Fig. 2, A). No serious burning was ob-

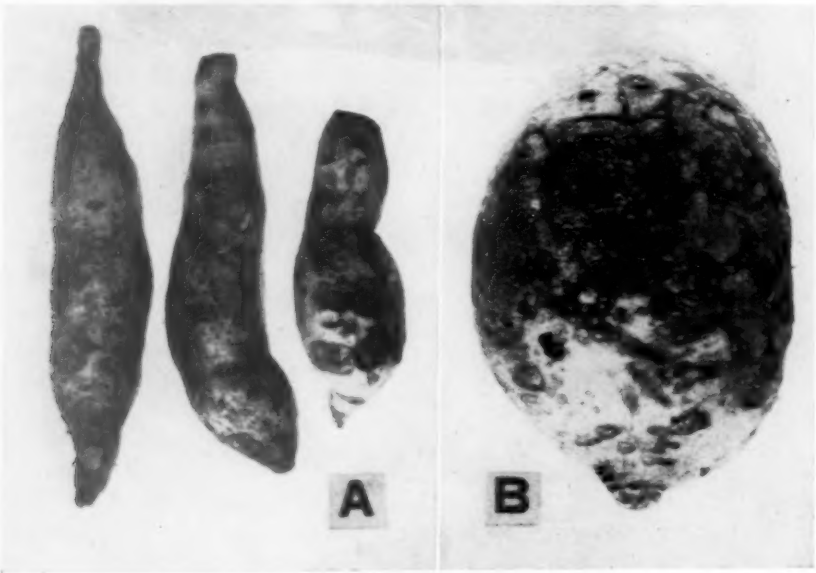

FIG. 2. Sweet potatoes showing chemical burn after treatment for control of black rot. A. Treated in a 2.5 per cent solution of borax. B. Treated in a 1.3 per cent solution of Dithane D–14.

served with any of the other concentrations of borax used. In the test with market potatoes the potatoes used were rather badly skinned and bruised at the time of treatment but even under these conditions the potatoes treated in 0.6 and 1.0 per cent solutions of borax showed no evidence of burning.

In the treatments with Dithane D–14, a rather severe chemical burning was noted on the bruised potatoes treated in 1.3 per cent solution (Fig. 2, B). Under laboratory conditions no evidence of burning was noted with concentrations up to 1.0 per cent of Dithane D–14.

BORAX RESIDUES

In order to have information concerning borax residues on treated sweet potatoes, Dr. E. A. Fieger of the Department of Biochemistry, Louisiana

State University, analyzed potatoes which had been dipped in solutions of borax at concentrations of 1.25 and 0.63 per cent. This was done to determine if borax in sufficient amounts adhered to the potatoes to make them unsafe for human consumption. The results of these analyses, which are included in table 3, indicate that boron residues are small.

TABLE 3.—*Results of analyses of sweet potatoes treated with borax*

Potatoes analyzed	Dry weight of boron (p.p.m.) in potatoes		
	Untreated	Dipped in 0.63 per cent solution of borax	Dipped in 1.25 per cent solution of borax
Raw (unwashed)			
Peelings	15.5	172.60	445.20
Flesh	4.8	4.40	6.70
Whole	4.8	18.40	33.20
Raw (unwashed) stored 20 days			
Peelings	16.28	157.90	285.70
Flesh	4.27	4.46	7.24
Raw (washed) whole			
In stream of water	4.80	17.50	22.20
With brush	4.80	13.40	22.40
Cooked (flesh)			
Baked	4.50	7.20	3.40
Peeled then boiled	3.70	7.00	4.90
Boiled in skins	4.20	6.30	5.30

SUMMARY

In Louisiana, black rot of sweet potatoes has increased in importance in the early crop which is washed and shipped to market before curing. In an attempt to find a fungicide treatment for use on these potatoes after the commercial washing process, 17 compounds were tested for their effectiveness in controlling black rot. The results of these tests indicate that there are definite possibilities for the commercial use of borax and perhaps Dithane D–14 at concentrations of approximately one per cent for controlling black rot on uncured sweet potatoes.

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INFLUENCE OF SOIL MOISTURE ON THE GROWTH OF THE POTATO PLANT AND ITS INFECTION BY THE ROOT-KNOT NEMATODE

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(Accepted for publication February 10, 1948)

The potato (*Solanum tuberosum* L.) has been grown as a commercial crop in the Territory of Hawaii since 1933, but not until the 1937–38 season was severe galling of tubers by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, noted. This season was outstanding in that rainfall was higher than usual and the soil remained wet for much of the time from December to April, when potatoes are produced in Hawaii.

Godfrey (1) showed that the amount of moisture in the soil seemed to play only a small part in root-knot development as long as the moisture content was favorable to the growth of crops. Little difference was found within the range of 40 to 80 per cent of the moisture-holding capacity of the soil; at 60, 70, and 80 per cent there appeared to be a slight increase over other moisture percentages but below 40 and above 80 per cent considerable knotting was found. Godfrey was of the opinion that temperature played a more important part than soil moisture in the development of *Heterodera marioni*. Gross (4) has reported an interesting observation: potatoes grown on land irrigated at frequent intervals showed less tuber galling than potatoes grown on land of equal infestation but irrigated less often. He believed that lowering the soil temperature from frequent irrigation inhibited the development of *H. marioni*. When controlled irrigation was tried there was but slight reduction in visible symptoms however, and daily irrigation of land caused a reduction in soil temperature of 2.7° F., compared with the temperature of soil irrigated at 5–day intervals.

The data presented here were obtained from greenhouse tests designed to determine whether galling of tubers is more severe in wet soils than in soils of lower moisture content, and to measure the reduction in yield of potatoes due to (a) inadequate soil moisture, (b) *Heterodera marioni*, and (c) a combination of these. Two separate experiments were conducted; one in which potatoes were grown in field soil that was infested, naturally and also artificially, with *H. marioni;* and another in which potatoes were grown in infested and fumigated soils.

EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF HETERODERA MARIONI IN POTATO TUBERS AND ROOTS

Materials and Methods

In this experiment, which lasted from August 1 to October 28, 1939, the

[1] Formerly Plant Pathologist, Hawaii Agricultural Experiment Station, Honolulu, Territory of Hawaii. Published with the approval of the Director as Scientific Paper No. 166, Hawaii Agricultural Experiment Station, Honolulu, T. H.

air temperatures in the greenhouse were 83° to 93° F. (weighted mean 87.7° F.) during the day, and 64° to 75° F. (weighted mean 70.5° F.) at night. For the two combined the weighted mean is 79.1° F., which is within the range reported by Godfrey (1) for gall formation. Soil collected from around the roots of field grown tomato plants affected with root-knot was mixed with finely chopped nematode infected roots of cucumber (*Cucumis sativus* L.) and bean (*Phaseolus vulgaris* L.). This soil was a reddish clay loam, of almost neutral reaction, with a field capacity moisture content of approximately 36 per cent, a moisture equivalent of approximately 32 per cent, and a wilting coefficient of approximately 24 per cent. The soil was well mixed daily for one week and kept moist. Commercial fertilizer considered adequate for normal growth of potato plants was incorporated, and an equal weight of soil was placed in each of 35 twelve-inch diameter clay pots previously weighed and counterbalanced. Their outer surfaces had been varnished to reduce evaporation. Several pieces of coarse wire screen were placed in the bottom of each pot for drainage. One potato tuber, 1.5–inch diameter, variety Bliss Triumph, outwardly free from nematode infestation was planted in each pot. Water was poured on the soil surface until leaching started, and also below the surface through 3 one-half inch diameter glass tubes whose lower ends were open and flared and placed 2, 4, and 6 inches deep, respectively. When leaching stopped the pots were weighed individually; although small differences in weight existed, the pots approximated 31 lbs. each. Soil moisture at this time, on an oven-dry basis, was 35 per cent, or almost the field capacity.

Fourteen pots were maintained at 31 lb. for 1 month by daily weighing and addition of water. The individual weights of 7 pots were allowed to fall to 29.5 lb., and those of the remaining 14 pots to 28 lb. by withholding water, which took about 1 week; these two weights were maintained for 1 month. For convenience in description, pots that weighed 31 lb. during the first month are referred to as the "Wet" series; pots that weighed 29.5 lb. during this time are referred to as the "Medium" series; and pots that weighed 28 lb. during the same period are referred to as the "Dry" series.

No claim is made in this experiment, or in experiment 2, that the moisture content of the soil in the series Wet, Medium, or Dry ever was held constant. Soil samples taken at intervals and at the end of the experiment showed that moisture in the soil probably fluctuated between 31.5 and 35 per cent in the Wet series, between 27.5 and 30 per cent in the Medium series, and between 24 and 27 per cent in the Dry series.

Seven pots were kept in each of the series (Wet, Medium, and Dry) for the duration of the experiment. Thirty days after planting 7 pots were transferred from the Wet series and 7 from the Dry series to the Medium series. To compensate for the growth of tops and roots, the maintained weight of every pot was increased by one-half pound. Sixty days after

planting, the 7 pots in the Medium series that had been transferred there at 30 days from the Wet series were placed in the Dry series, and the 7 originally in the Dry series were placed in the Wet series. When the first change in soil-moisture was made (30 days), plants previously in the Wet series when changed to the Dry series wilted, particularly during the day, but recovered at night. Wilting was less marked, or absent, when plants previously in the Wet series were changed to Medium. After about 5 days no wilting was observed.

Results

All plants when 30 days old were measured for height; those in the Wet series averaged 11.7 cm., those in the Medium averaged 8.9 cm., and those in the Dry were 7.3 cm.

In table 1 a summary is given of the relative vigor of tops of the potato plants, at 60 days, in relation to the different moisture treatments. From the "mean vigor scores" it can be seen that most vigorous growth was

TABLE 1.—*Relative vigor of potato plant tops in relation to soil moisture, after 2 months*

Soil moisture [a]	Number of plants per vigor class				Mean vigor score [b]
	Very good	Good	Poor	Very poor	
WW	6	1	0	0	3.9
MM	0	4	3	0	2.6
DD	0	2	2	2	2.0
WM	3	3	1	0	3.3
DM	0	4	1	2	2.3

[a] W = wet, M = medium, D = dry; the 2 letters represent moisture conditions for consecutive 30-day periods. Seven plants were in each group, but one plant in DD was lost through accident.

[b] Vigor scores: 1 = very poor, 2 = poor, 3 = good, and 4 = very good.

obtained when the soil was continuously wet (WW) and poorest when it was continuously dry (DD), with a somewhat intermediate figure when the moisture was moderate in its amount (MM). Growth was better in the WM series than in the DM, MM, or DD series.

Plants were harvested as soon as the tops died, usually 70 to 82 days after planting, with a few instances of 85 to 89 days. There was no evidence that the potato plants were attacked by organisms other than *Heterodera marioni*. Tubers, underground stems and stolons, and roots were washed free of soil, the tubers weighed, and tubers and roots examined, outwardly and microscopically, for *H. marioni*. Infection generally was heavy.[2] Data are summarized in table 2. Yields were highest in the WWW series, that is, with the soil kept continuously well moistened; but accompanying the high yields was severe galling of tubers and often of roots. Fifty per cent less yield, but with less galling of tubers and roots

[2] Eighteen Whippoorwill cowpea plants, 3 growing for 30 days in each of 6 small cans, each can containing approximately 650 grams of the soil in which the potatoes were planted had an average of 616 nematode galls per plant on the root systems.

and lowest mean severity scores, was obtained in the WMD series, in which the soil moisture was lowered progressively as the plants got older. Galling of tubers was less in moisture series MMM and DDD than in the WWW series, with most severe galling of roots in series DDD. Somewhat confirming the trend is the result found in series DMW, where galling of tubers was more severe than in series WMD. Findings in experiment 1 appear to show that tuber galling is more severe in wet than in dry soil, while root galling is more pronounced in dry than in wet soil.

TABLE 2.—*Longevity, yield, and severity of attack by Heterodera marioni of potato plants in infested soil when held at relatively constant levels of moisture or varied at monthly intervals*

Soil moisture [a]	Av. life of plants (days)	Av. yield (gm.)	No. plants with tubers		Mean severity scores	No. plants with roots		Mean severity scores [b]
			Severely galled	Slightly galled		Severely galled	Slightly galled	
WWW	81	320	7	0	2.0	5	2	1.7
MMM	71	100	4	3	1.5	5	2	1.7
DDD	70	24	4	2	1.6	6	0	2.0
WMD	79	161	2	5	1.2	2	5	1.2
DMW	70	95	7	0	2.0	6	1	1.8

[a] W = wet, M = medium, D = dry; the 3 letters represent moisture conditions for consecutive 30-day periods. Seven plants were in each group, but one plant in DDD was lost through accident.

[b] Mean severity scores: 1 = slightly galled, 2 = severely galled. Mean severity score represents sum of plants in each severity class times the score for that class divided by the total number of plants.

EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF HETERODERA MARIONI ON POTATO AND ON NORMAL GROWTH OF THE PLANT

Materials and Methods

To better interpret the results of the first experiment a second was planned. Potatoes were grown in nematode-infested and in fumigated soil and the effect of differences in soil moisture on the growth of the plant and on the activities of *Heterodera marioni* determined. The same soil type was used as in experiment 1 and it was handled similarly except that it was infested with peelings from the nematode-infected potato tubers from experiment 1. Enough soil was infested to later fill 60, twelve-inch diameter clay pots, varnished on their outer surfaces. Commercial fertilizer adequate for normal growth was mixed with the soil. Half of the soil was fumigated 5 days with chloropicrin (2.5 cc. per cubic foot) in closed containers, spread out on tables in the greenhouse for 5 days to allow escape of the nematocide, then well wetted, and placed in 30 pots. Pots were counterbalanced as before, an equal weight of soil placed in each, and one

potato tuber (Bliss Triumph), outwardly free of nematode infection, planted per pot. The soil was watered until seepage started; when it stopped the pots plus soil were weighed. Experiment 2 lasted from November 8, 1939, to February 5, 1940, when the greenhouse day temperatures varied from 74° to 99° F. (weighted mean 87.6° F.) and the night

TABLE 3.—*Longevity, yield, and severity of attack by Heterodera marioni of potato plants in infested and also in fumigated soil when held at relatively constant soil moistures or soil moistures varied at monthly intervals*

Soil treatment and soil moisture [a]	Age of plants when harvested (days)	Av. yield (gm.) [b]	No. plants with tubers		Mean severity scores [c]	No. plants with roots		Mean severity scores [c]
			Severely galled	Slightly galled		Severely galled	Slightly galled	
Fumigated								
WWW	30	0	...	...	.....	0	0	0.0
	60	368	0	0	0.0	0	0	0.0
	90	550	0	0	0.0	0	0	0.0
DDD	30	0	...	...	.....	0	0	0.0
	60	214	0	0	0.0	0	0	0.0
	90	297	0	0	0.0	0	0	0.0
WMD	60	266	0	0	0.0	0	0	0.0
	90	388	0	0	0.0	0	0	0.0
DMW	60	200	0	0	0.0	0	0	0.0
	90	410	0	0	0.0	0	0	0.0
Infested								
WWW	30	0	...	...	.....	0	2	0.6
	60	146	2	1	1.6	0	3	1.0
	90	324	2	1	1.6	1	2	1.3
DDD	30	0	...	...	.....	0	1	0.3
	60	57	1	1	1.5	2	0	2.0
	90	152	1	2	1.3	3	0	2.0
WMD	60	180	1	1	1.0	0	3	1.0
	90	222	2	1	1.6	1	2	1.3
DMW	60	165	1	2	1.3	2	1	1.6
	90	245	3	0	2.0	2	1	1.6

[a] W = wet, M = medium, D = dry; the 3 letters represent moisture conditions for consecutive 30-day periods. Three plants were harvested at 30, 60, and 90 days, respectively, from each group except at 60 days in infested soil, group DDD, where one plant was lost through accident.

[b] Variation in yield between individual plants was less than 10 per cent; a yield of 0 indicates that no tubers were present.

[c] Mean severity scores: 0 = not galled, 1 = slightly galled, 2 = severely galled. Mean severity score represents the sum of plants in each severity class times the score for that class, divided by the total number of plants.

temperatures from 57° to 74° F. (weighted mean 65.4° F.); the weighted mean for all temperatures combined is 76.5° F., within the range reported by Godfrey (1) for gall formation.

The two extremes of soil moisture, Wet (W), and Dry (D), were studied; and in a few pots the soil was held at the Medium (M) moisture during the second month of the plants' growth. Pots in the Wet series were maintained at 34.5 lb. for 30 days after planting, increased to 35 lb. for the next 30 days, and after 60 days increased to 35.5 lb. In the Dry

soil-moisture level, saturated soil was allowed to dry out until weight of pot plus soil was 31 lb., which took about 5 days; the pots were maintained at 31 lb. for 30 days after planting, increased to 31.5 lb. for the next 30 days, and after 60 days increased to 32 lb. Medium soil moisture was obtained by keeping the combined weight of pot plus soil at 32.5 lb. The percentage of soil moisture in the Wet series on an oven dry basis varied from 32 to 36 per cent as shown by testing samples taken from various pots, and from various parts of the soil within individual pots, at intervals during the course of the experiment. In the Medium moisture series the soil moisture varied from 29 to 33 per cent, and in the Dry series the variation was 24 to 30 per cent. Four moisture schedules were followed: (a) Eighteen plants in soil continuously Wet (WWW); (b) Eighteen plants in soil continuously Dry (DDD); (c) Twelve plants in soil that was Wet for 30 days, moderately moist (M) for the next 30 days, and Dry for the next 30 days (WMD); and (d) Twelve plants in soil with moisture conditions the reverse of "c" (DMW). Half of the pots in each of these 4 schedules was filled with nematode-infested soil and the other half with fumigated soil. Pots were weighed daily and returned to desired weights by addition of water. Six plants, 3 growing in nematode-infested soil and 3 growing in fumigated soil, were harvested at 30, 60, and 90 days after planting, respectively, from moisture schedules WWW and DDD. Sixty days after planting, and again at 90 days, 6 plants, 3 growing in infested soil and 3 growing in fumigated soil, were harvested from moisture schedules WMD and DMW, respectively (Table 3).

Roots, tubers, and underground stolons were washed free of soil, and the tubers weighed. After macroscopic examination, the roots were discarded. Tubers next were examined, macroscopically and microscopically, for nematode infection; no infection was found on underground parts of plants grown in fumigated soil. Data are summarized in table 3.

Results

Highest yields were obtained in continuously Wet (WWW) fumigated soil and lowest in infested Dry (DDD) soil, at both 60 and 90 days. Plants harvested at 30 days bore no tubers. At 60 days, yields in Dry soil were reduced 42 per cent, compared with an 85 per cent reduction in infested Dry soil and a 60 per cent reduction in infested Wet Soil. At 90 days there was little difference in yield in a fumigated Dry soil and a Wet soil infested with *Heterodera marioni;* in the Wet soil the nema reduced yields by 41 per cent and in the Dry soil the reduction was 48 per cent. The lower the soil moisture the greater the reduction in yield attributable to *H. marioni.*

When the soil moisture was varied, results were not always so clear cut. In fumigated soil, moisture schedule DMW produced 24 per cent less tubers at 60 days than WMD and about the same as WMD at 90 days, but at

neither date did either DMW or WMD produce the yield obtained in WWW soil. In infested soil there was little difference between yields, at 60 or 90 days, in schedules WMD and DMW, and again neither produced the yields obtained in WWW soil (infested or fumigated). To obtain greatest yields in the presence of *Heterodera marioni* it seems necessary to have the soil moisture as high as possible.

Tubers were not attacked so severely as in experiment 1, possibly due to slightly lower air and soil temperatures. Infection was adequate, however, to show that tubers grown in wet soil are attacked more severely by *Heterodera marioni* than tubers grown in dry soil (Table 3); for tubers of the same size, from wet and from dry soil, there were more and larger galls on the former. In contrast, more root galling was encountered in dry infested soil, which was thought to be due to infection by *H. marioni*. No counts of galls per root system were attempted. These findings confirm those obtained in experiment 1.

Tubers produced in wet soil under field conditions usually have enlarged lenticels. In experiment 2 enlarged lenticels were found in fumigated and also in infested wet soil. Only by microscopic examination could the plant morphology, lenticel, lenticel-gall, or gall be determined with certainty. Freehand sections through the enlargements, killed and fixed in chrom-acetic acid and stained with osmic acid (2), sufficed to reveal the presence or absence of the nematode[3]. Lenticels seem to serve as avenues of entrance for larvae of *Heterodera marioni*, and it was easy to find larvae just beneath the cambial layer of enlarged lenticels, obviously recent infections. Large galls that had been originally lenticels were also observed, with the nematode, in various stages of development, lying directly opposite the natural opening of the epidermis of the tuber. That enlarged lenticels serve as avenues of entrance for *H. marioni* has not been pointed out before. Enlarged lenticels on tubers produced in wet fumigated soil showed no nematodes, and at the time it was not possible to examine lenticels of normal size for nematode infection.

DISCUSSION

These results are based on a relatively small number of plants in each series, but the nature of the research called for expending considerable time in handling the plants and a greater number, while desirable, was not possible. The method of adding water to the soil is open to criticism; each time water is added the wet soil condition is established locally, permitting the nematode and contaminating fungi to overcome any temporary limitations imposed by previous drought. Differences in soil moisture were obtained, however, the "wet" soil differed considerably from the "dry" soil, and to obtain these contrasts was the endeavor of the research. The

[3] No substitute for the expensive osmic acid was found though much time was spent trying alkannin, Sudan III, cotton blue, acid fuchsin, and scarlet R, the last 3 of which are recommended by Goodey (3) for differentation of *Heterodera marioni* in root and stem tissue. They did not work in potato tuber tissue.

nematode *Heterodera marioni* seems to injure potato tubers more in wet soil than in dry soil, certainly their sales value is lessened, but it also seems to cause less injury to roots in wet than in dry soil. Roots were galled severely, sometimes even rotted and decaying, in dry infested soil, whereas in wet infested soil the root systems seemed more capable of competing with the attacks of the nematode. It is well known that presence of moisture in the soil is an inducement to root formation. What are the relative speeds of root galling and new root formation in a wet soil and in a dry soil? If the nematode can attack and gall roots as soon as they are formed then the nematode will be dominant. If, however, a plant can produce roots faster than the nematode can attack them, the general picture will be one of a plant with functioning roots not suffering particularly from attack by *H. marioni*. Detailed research is needed, but the results obtained here indicate That in nematode infested soil, for good yields, the potato plant needs abundant soil moisture although galling is more severe and noticeable in wet soil than in drier soil. In dry soil the tuber galling is less than in wet soil but yields are low, probably lower than commercially desirable.

The evidence presented here is not in agreement with the observation by Gross (4) that potatoes grown on land irrigated at frequent intervals had less tuber galling than potatoes irrigated less, but a comparison between the two locales is hardly justified because too many other factors are operating. In fact, Gross admits that controlled irrigation resulted in only a slight reduction in visible symptoms; even in the same location it was difficult to duplicate results. These present data tend to confirm field results of the 1937–38 potato growing season in the Hawaiian Islands, namely, galling of tubers is more severe when the soil is extremely wet than in years of normal precipitation. The difficulty experienced in separating an enlarged lenticel and a nematode gall, on tubers grown in wet soil, raises an interesting question. Were all of the "galls" on the tubers in the 1937–38 potato crop of the Hawaiian Islands actually due to attacks by *H. marioni*, or were some, possibly a majority, merely enlarged lenticels? Regardless of their true nature, the tubers were of unsatisfactory appearance and growers were penalized and lost money thereby.

SUMMARY

Potatoes grown in Hawaii in 1937–38 were galled severely by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, in a growing season distinguished from others because of excessive rainfall which kept the soil wet for much of the growing season, December to April.

Potatoes were grown experimentally in nematode-infested and in chloropicrin-fumigated soil of varying moisture content, wet soil in which the moisture probably fluctuated between 31 and 36 per cent, in dry soil with moisture content of 24 to 27 per cent, and in a soil of intermediate moisture content, 27 to 32 per cent. No claim is made that the moisture content was ever held constant, but it was controlled within certain limits.

The higher the soil moisture, the higher the tuber yields in fumigated as well as in infested soil, but the higher the soil moisture the greater the injury to tubers produced in an infested soil through galling caused by the parasite *Heterodera marioni* (Cornu) Goodey. In the presence of the nematode and in a dry soil, yields were lower than commercially profitable, therefore the soil moisture should be adequate if the root-knot nematode is present. Nematode damage to roots seemed to be greater in dry soil than in wet soil.

Evidence was found that the root-knot nematode can enter potato tubers through enlarged lenticels, which are commonly observed on tubers grown in wet soil. This is believed to be the first time this type of infection has been found. No opportunity presented itself to study the possible entry of *Heterodera marioni* into tubers bearing normal lenticels.

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INTERRELATION OF BEAN VIRUS 1 AND BEAN VIRUS 2 AS SHOWN BY CROSS-PROTECTION TESTS

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Bean virus 1 and *bean virus 2,* the incitants of common mosaic and yellow mosaic, respectively, of bean (*Phaseolus vulgaris* L.) have physical properties which are practically identical (8). They differ primarily in host range, symptoms, and seed transmission. *Bean virus 1* does not infect ordinarily a considerable list of varieties of *Phaseolus vulgaris* while some strains of *bean virus 2* infect all varieties tested so far. *Bean virus 1* affects a narrower range of other legumes than does *bean virus 2.* In general the symptoms produced by *bean virus 2* are different and often more severe than those produced by *bean virus 1. Bean virus 1* is transmitted in bean seed. *Bean virus 2* is not reported, so far, to be seed-borne.

Inasmuch as the differences cited above are no greater than those now commonly recognized between strains of a single virus, the question arose as to whether these viruses should be regarded as distinct or as strains of a single virus. This question becomes the more pertinent since more than one strain of each virus has been observed. The present investigation was concerned with the cross-protection effect of one virus when inoculated in the bean plant in advance of the other.

MATERIALS AND METHODS

Stock cultures of *bean virus 1* were maintained in Stringless Green Refugee plants grown from virus-free seed in the greenhouse. *Bean virus 2* was maintained in plants of Sensation Refugee 1066 also grown from seed in the greenhouse. Use of the latter variety practically eliminated the possibility of a mixture with *bean virus 1* since it is highly resistant to the latter virus. The greenhouses in which studies were conducted were kept free of insects by frequent fumigation and spraying. The experimental plants were grown at temperatures usually ranging from 24° to 28° C. In all instances, unless otherwise stated, inoculations were made by rubbing with carborundum as an abrasive.

Bean virus 1 was obtained from infected seed material supplied by Dr. W. J. Zaumeyer. This virus caused leaf curling and mottle on susceptible varieties and occasionally caused the production of hydrotic or greasy pods. These symptoms agree with those reported by other workers for this virus (2, 4). On varieties possessing resistance derived from Corbett Refugee (1, 7, 9, 10, 12), it caused systemic necrosis on young plants and symptoms of black root on plants in the pod stage (5, 6). The culture of *bean virus 2* used was secured from naturally infected plants in the vicinity of Madison, Wisconsin, and is believed to be similar to the strain used by Pierce (8). It was infectious to all varieties of bean tested.

Sensation Refugee 1066 and Idaho Refugee seed were supplied by Dr. M. E. Anderson of the Rogers Bros. Seed Co. Stringless Green Refugee seed was grown at Madison in isolated plots which were carefully rogued for common bean mosaic.

Plants of Stringless Green Refugee, which is susceptible to both *bean viruses 1* and *2*, were used as test plants. The methods of conducting the cross-protection tests and methods of assay for the presence of the two viruses are described in connection with the presentation of the experimental results.

EXPERIMENTAL RESULTS

Specific Protection Against Bean Virus 2 Produced by Bean Virus 1

Cross-protection tests involving *bean virus 1* and *bean virus 2* were set up as follows. Each virus was inoculated to 24 Stringless Green Refugee bean plants. Nine days later when these plants had developed systemic symptoms, the *bean-virus-1*-infected plants were inoculated with *bean virus 2* and the *bean-virus-2*-infected plants were inoculated with *bean virus 1.* At the time of the second inoculation 10 healthy plants of the same age were inoculated (to serve as controls) with *bean virus 1* and 10 with *bean virus 2.* Ten days after the second inoculation practically all plants of the control groups showed symptoms characteristic of common and yellow mosaic, respectively. Test plants which were first inoculated with *bean virus 1* and later with *bean virus 2* were evidently infected with the first virus only, since they showed none of the symptoms usually caused by *bean virus 2.* However, some test plants which received the reverse treatment (*bean virus 2* followed later by *bean virus 1*) had apparently become infected with *bean virus 1* as evidenced by rolling and cupping of the young leaves, a characteristic symptom of common mosaic. To determine the presence or absence of the two viruses in the test plants, leaves from each group of plants were ground separately in a sterile mortar and inoculations were made to four plants each of Stringless Green Refugee and Sensation Refugee 1066. Both viruses could infect the former, but Sensation Refugee is highly resistant to *bean virus 1* and therefore only *bean virus 2* would develop in plants of this variety. All Stringless Green Refugee plants inoculated with juice from the group inoculated with *bean virus 1* alone, or with this virus first and *bean virus 2* later, became infected with *bean virus 1.* Sensation Refugee plants inoculated with a part of the same juice remained symptomless except for an occasional plant which developed systemic necrosis caused by *bean virus 1* (3). Stringless Green Refugee assay plants inoculated with juice from test plants inoculated with *bean virus 2* first and *bean virus 1* later became infected with *bean virus 1* in 13 of 24 cases. All Sensation Refugee plants inoculated with a portion of the same juice became infected with *bean virus 2.* This experiment was repeated three times with essentially the same results.

Seed of Stringless Green Refugee was saved from plants of the group inoculated first with *bean virus 2* and later with *bean virus 1*, and from con-

trol plants inoculated with *bean virus 1* alone. This seed was planted in the greenhouse and observed for the occurrence of common mosaic in an attempt to determine the effect of *bean virus 2* on the seed transmission of *bean virus 1*. Results (Table 1) show that when *bean virus 2* became established in a plant it reduced the amount of seed transmission of *bean virus 1*. However, since the number of seed used was small these results were considered as indicative but not conclusive.

In an experiment conducted in the same way as the one just described, except that the second inoculations were made 6 days instead of 9 days after the first, practically no cross-protection occurred. This raised the question as to how long each virus must be present in the plants before one protected completely against the other. An experiment was set up in which the second inoculations were made 6, 8, 10, 12, and 14 days, respectively, after the first. Eight plants were used for each inoculation interval and for each virus the same number of healthy plants of the same age were inoculated to serve as controls. An assay was made as described in the previous experi-

TABLE 1.—*The effect of bean virus 2 on seed transmission of bean virus 1*

History of seed plants	Seed Planted	Total Plants	Plants infected with *bean virus 1*	
			Number	Percentage
	No.	*No.*		
Inoculated with *bean virus 2* followed by *bean virus 1*	19	16	3	19
Inoculated only with *bean virus 1*	36	20	12	60

ment. When plants inoculated with *bean virus 1* were inoculated with *bean virus 2* 6 and 8 days later, very little protection occurred, but when inoculations were made at 10, 12, and 14 days no development of yellow mosaic occurred. *Bean virus 2* protected against *bean virus 1* completely at 12 and 14 days. *Bean virus 2*, therefore, required a slightly longer period than did *bean virus 1* to protect against the opposite virus.

As reported elsewhere (3), when plants of varieties in which resistance to *bean virus 1* had been derived from Corbett Refugee were inoculated with *bean virus 1* through approach grafts to Stringless Green Refugee, they showed necrosis and death characteristic of black root. Since *bean virus 2* protected against *bean virus 1* in other tests, it was of interest to determine whether it could protect against necrosis caused by *bean virus 1* if the Stringless Green Refugee portions of the grafts were inoculated with *bean virus 2* before they were inoculated with *bean virus 1*. Approach grafts were made between healthy Stringless Green Refugee and Idaho Refugee plants. Thirteen grafts were inoculated with *bean virus 2* and 13 were left noninoculated. Nine days later, when yellow mosaic symptoms had developed in both parts of the 13 grafts inoculated with *bean virus 2*, they were inoculated with *bean virus 1*. The 13 grafts which were not inoculated with *bean*

virus 2 were treated as follows: seven were inoculated with *bean virus 1*, three with *bean virus 2*, and three were left noninoculated. Results of these inoculations are presented in table 2. The Idaho Refugee portion of five grafts inoculated with *bean virus 1* alone died within a short time after inoculation. Twelve of 13 grafts which had been inoculated with *bean virus 2* before being inoculated with *bean virus 1* developed a mottle typical of yellow bean mosaic but did not become necrotic, and at maturity no root necrosis was evident.

TABLE 2.—*The prevention of systemic necrosis caused by bean virus 1 in Idaho Refugee plants by earlier inoculations with bean virus 2*

Treatment	Total grafts	Grafts which became necrotic	
	No.	No.	Percentage
Inoculated with *bean virus 2* followed by *bean virus 1* ten days later	13	1	7.7
Inoculated with *bean virus 1* at time of second inoculation above	7	5	71.4
Inoculated with *bean virus 2* at same time as above	3	0	0.0
Noninoculated	3	0	0.0

DISCUSSION

Most studies of cross protection are based on local-lesion-mottle tests. However, such a reaction is not necessary for a successful immunity test. Pound and Walker (11) state "where two related viruses have a differential reaction on any host their ability to immunize plants against each other can be tested in any plant in which they develop systemically." The correctness of this statement is borne out by this study with *bean viruses 1* and *2* which do not develop local lesions on any host in so far as known, but which lend themselves readily to tests for specific immunity because of their differential reactions on certain bean varieties. It was assumed that if one of the viruses by earlier inoculation could prevent the development of the second virus to such an extent that it could not be recovered by inoculation back to a susceptible variety, that specific immunity had developed. On this basis, it has been shown in this study that specific immunity was developed by *bean virus 1* to *bean virus 2* and vice versa. Plants of Idaho Refugee infected with *bean virus 2* and inoculated with *bean virus 1* by approach graft with Stringless Green Refugee became necrotic in one out of 13 cases while in plants not previously infected with *bean virus 2* five out of seven became necrotic when infected with *bean virus 1*. Limited evidence was presented which indicates that *bean virus 2* caused a reduction in the amount of seed transmission of *bean virus 1*.

Previous workers have shown that *bean virus 1* and *bean virus 2* do not differ appreciably in dilution end point, thermal inactivation, and longevity

in vitro. The only points of difference are in symptoms produced, seed transmission, and host range. The fact that they effectively cross-protect against each other in bean is further evidence that they are closely related viruses.

SUMMARY

By the use of the differential reaction to *bean virus 1* and *bean virus 2* in Stringless Green Refugee and Sensation Refugee 1066 bean varieties, the former virus was shown to effectively immunize beans against infection with the latter and likewise *bean virus 2* was shown to partially or entirely immunize beans against *bean virus 1*. *Bean virus 2* also reduced the amount of seed transmission of *bean virus 1* and reduced the amount of systemic necrosis caused by *bean virus 1* on certain resistant varieties. In view of the facts that they are very similar in dilution end point, thermal inactivation, longevity in vitro and that they protect against one another in bean, they are regarded as closely related viruses. The chief differences are in host range and in the fact that *bean virus 1* is transmitted in bean seed while *bean virus 2* is not.

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THE GENERIC AND SPECIFIC CHARACTERS OF PHYTOPATHOGENIC SPECIES OF PSEUDOMONAS AND XANTHOMONAS

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Many of the descriptions of the bacterial plant pathogens, especially of those forms occurring in the genera now known as *Pseudomonas* and *Xanthomonas,* have appeared identical except for the characteristic of pathogenicity. Consequently, it has been impossible to identify these bacteria successfully by means of the usual procedures of the bacteriological laboratory, and the question has arisen whether or not there are numerous synonyms present. The differences in pathogenicity possibly preclude this supposition and, where careful comparisons have been made, relatively few synonyms have been shown to exist among the phytopathogenic bacteria. One might argue that there are a limited number of true species among the phytopathogenic bacteria in these genera, and that a great many *formae speciales* exist, if one prefers to use this mycological term. This is probably not the entire story either, and, in order to investigate this question, we have taken, as our working hypothesis, the belief that where there is a true difference in pathogenic ability, some other type of difference should be demonstrable—be it cultural, biochemical, metabolic, serological, or of some other category. We do not infer that such differences necessarily explain the differences in pathogenicity.

One of the reasons for the many identical descriptions in these genera is the failure of the investigator to recognize which bacteriological characters are generic, and which are specific. Only too frequently, the descriptions of many bacterial plant pathogens offer only generic characters. We are referring to the general run of descriptions that on the surface appear adequate, not to the large list of named plant pathogens that has been placed in appendices of Bergey's "Manual of Determinative Bacteriology," because the described characters of these are too few and too confused for one to be certain of the species. In the present paper, the writers are endeavoring to point out what characters are generic, and what characters might be used in separating the phytopathogenic species for the genera *Pseudomonas* and *Xanthomonas.*

In making this report, we are not proposing a final scheme and key to the species, nor do we consider the minutiae of our results absolutely conclusive since there was not available, in all cases, a sufficient number of isolates to assure statistical validity. However, the results do indicate means of distinguishing some of the bacteria, and, what is perhaps more important, point the way toward the likelihood that further study will uncover additional means of separation.

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METHODS

The cultures which were studied represent a collection which was assembled over the years for a variety of purposes (20, 22, 23). In the majority of cases, they are cultures which the writers isolated and proved pathogenic at the time of isolation. In some instances, the bacteria were received from other investigators, who were working with those species, and whose identifications we had reason to consider authentic.

The search for specific differences at times involves the trial of techniques and criteria which are not at present in routine use for this group of bacteria. The experimental methods used in the present investigation to separate species of phytopathogenic bacteria are now given.

Tolerance of sodium chloride. Salt-tolerance of phytopathogenic bacteria has received comparatively little systematic study. In some cases, the NaCl-tolerance of individual species is included in the descriptions, but the conditions under which this characteristic was determined varied with the laboratory to an extent which prohibits comparison of the results given by different workers. In the present study, the following procedure was used. A series of salt broths was prepared using as a base 0.3 per cent of Bacto yeast extract and 0.5 per cent Bacto tryptose in distilled water, with the NaCl concentration ranging from 1 to 10 per cent in one per cent steps. The reaction after autoclaving was pH 7.0 to brom thymol blue and the media were used before evaporation took place. In order to avoid variations in salt-tolerance which occur as a function of the age of the bacterial cells being tested (2, 14) uniform loopsful of 48-hour-old cultures in yeast extract-tryptose broth without added NaCl were used as inoculum. The turbidity that developed in the several salt concentrations, and in the control medium without added salt, was examined visually every day for a week and, in the case of a few slow-growing organisms, for two weeks.

Tyrosinase activity. Some phytopathogenic bacteria color the ordinary peptone media brown. Certain species are especially active in this respect (3, 4, 5, 15, 16, 17) and the suggestion has been made that the brown coloration be used as a taxonomic character or as an aid in isolating actively browning bacterial species from infected plants. Other than such incidental observations, little attention has been devoted to the problem; nor have adequate explanations been offered of the mechanisms whereby phytopathogenic bacteria produce these colorations. Indeed, there is a tendency to assume, without direct experimental evidence, that any browning of culture media is a "tyrosinase reaction"—actually an oxidation and polymerization of the amino acid tyrosine to dark melanin pigments.

Tyrosinase tests of *Pseudomonas* isolates were made in a liquid asparagin-glycerol-tyrosine medium prepared by omitting agar from the formula given by Starr (19). The *Xanthomonas* species were tested in liquid media prepared by adding 0.05 per cent tyrosine to a solution (20) which satisfies the minimal nutritive requirements of each species. The tyrosine medium and a tyrosine-free control medium were inoculated from young agar slant

cultures and incubated at 28° C. Cultures were observed periodically for growth and for pigmentations of the medium. A culture was considered tyrosinase-positive if it grew in both media, and produced, only in the tyrosine medium, a red color which eventually became dark brown.

Some of the cultures were tested, also, on an agar tyrosine medium (19). In general, the cultures which browned the liquid medium also browned the agar medium. In addition, a number of strains which appeared tyrosinase-negative in the liquid medium were able to make distinct red-to-brown colorations of the agar medium. Inasmuch as these differences cannot at this time be explained, and since the results in liquid media show promise of utility in determinative bacteriology, the data obtained from experiments in solid media will not now be presented in full or discussed further.

Liquefaction of sodium ammonium pectate. The ability of phytopathogenic bacteria to decompose pectic substances has never been systematically studied. The scattered literature reports experiments with a variety of ill-defined "pectin" preparations, and it is likely that some of the workers in this field had not realized the heterogeneous nature of pectic substances or of pectic enzymes (12, 13). Following Jones' pioneer study (11), the soft-rotting of fresh tubers and fleshy roots has been considered by many investigators as synonymous with pectinolytic activity, although this supposition is not always proved. One of the reasons for the lack of knowledge in this field has been the unavailability of pectic substances which lend themselves to routine microbiological use. A preparation of sodium ammonium pectate has recently become available,[3] which, with the addition of a calcium salt, can be made into media similar to gelatin and can be sterilized by autoclaving. The properties and use of this material are given by Baier and Manchester (1) and by Starr (21). We have prepared media of this pectate, with and without the addition of peptone or yeast extract, and have cultured phytopathogenic bacteria in these media. If the pectate is utilized, a liquefaction takes place. We do not claim, however, that organisms that liquefy this medium are necessarily able to attack the pectic substances of the middle lamella of all plant cells.

Utilization of sodium tartrate. The utilization of sodium tartrate as the sole carbon source had been shown, in preliminary experiments, to be of some differential value for the phytopathogenic species of *Pseudomonas.* Observations on a more extensive series of bacteria are now reported. The sodium tartrate was supplied at a concentration of 0.15 per cent in the synthetic medium of the "Manual of Methods for Pure Culture Study of Bacteria" (7). This medium was seeded heavily, incubated, and examined for growth and alkali production at intervals up to 2 weeks. These experiments are reported with the realization that possible growth factor deficiencies and toxicity of the tartrate were not controlled, and that "failure to grow" may actually result from one of these factors rather than from unsuitability of tartrate as carbon source. On the other hand, as will be seen

[3] Product 24 of California Fruit Growers Exchange, Ontario, California.

below, the medium, when used as specified herein, does give results of differential value.

Reduction of nitrates. The reduction of nitrates to nitrites has been used extensively in the past with varying results. Before attempting to employ this reaction one should read Conn's suggestions (8) and avoid the many pitfalls he lists. In the present investigation, the bacteria were grown from large inocula in the synthetic nitrate medium described in the "Manual of Methods for Pure Culture Study of Bacteria" (7). Tests were made at the end of 2 days and at various times up to 2 weeks, for both nitrites and nitrates using the reagents recommended in the manual. The experiment was repeated three times. The species also were grown in beef extract-peptone medium plus KNO_3 and tested for nitrites. This latter medium is not so reliable as the synthetic medium because in many cases where a reduction was readily demonstrated in the synthetic medium it was not demonstrated in the beef extract-peptone broth. The latter medium, however, supported better growth. One series of tests also was made for ammonia in the synthetic solution where the *Pseudomonas* species were being grown, but the only positive reaction obtained was with *P. caryophylli.*

PSEUDOMONAS

The genus. The phytopathogenic members of *Pseudomonas* have been characterized in Bergey's "Manual of Determinative Bacteriology," but a description of this group is desirable at this point in order to indicate the generic characteristics of the plant pathogens. A member of this genus is a medium-sized rod, Gram-negative, with 1–6 polar flagella, and without spores. It turns milk alkaline; produces no indol nor H_2S; utilizes monosaccharides but not as a rule the higher carbohydrates with the exception of sucrose. Starch is not hydrolyzed. Most species can utilize natural asparagine as the sole carbon and nitrogen source. These bacteria are aerobic, or facultatively anaerobic with optimum temperatures at 27°–30° C. The species remain viable on beef extract–peptone agar for many months but not on potato-dextrose agar.

The species. What characters can be used to separate the species in the genus *Pseudomonas?* At present, not many have been studied, but those which are known should be stressed, and tests to determine them in all instances should be carefully conducted. Clara (6) pointed out that the plant pathogens in the genus *Pseudomonas* could be separated into two groups; those that can utilize sucrose and those that cannot. The character appears to be a reliable one and, since many of the species of *Pseudomonas* grow readily in a simple synthetic solution, it can be determined easily. Where a growth factor is required it may be added, but unless purified chemicals and glassware are used it will probably not be necessary. Filtered sugars are recommended.

Gelatin liquefaction is a second specific character of importance on which the genus may be subdivided and is one usually recognized. It is easily determined, and as a rule is used in all descriptions.

The ability to reduce nitrates to nitrites, ammonia or nitrogen, while it had not been neglected with these pathogens, had rarely been very carefully determined. It is thought by certain investigators that all the *Pseudomonas* species reduce nitrates, but our work does not entirely substantiate this belief. However, some of the pathogens that have been described as non-reducers have been shown in this investigation actually to possess this ability.

The following species were found by us to be able to reduce nitrates to nitrites: *Pseudomonas aceris, P. alliicola* [5],[4] *P. angulata* [3], *P. apii* [2], *P. aptata, P. atrofaciens* [2], *P. barkeri, P. berberidis* [2], *P. caryophylli* [2], *P. delphinii* [2], *P. glycinea* [3], *P. lachrymans, P. lapsa, P. mori* [3], *P. papulans, P. savastanoi* var. *fraxini, P. syringae* [11], *P. tabaci* [9], *P. tomato* [3], *P. tonelliana, P. viridilivida, P. washingtonia.*

Pseudomonas striafaciens and *P. viburni* [2] gave very weak nitrite reactions, but no nitrites were detected in the tubes of any of the remaining cultures which were used for this and most of the other experiments described herein: *Pseudomonas cichorii, P. coronafaciens* [2], *P. maculicola, P. marginata* [2], *P. mellea, P. phaseolicola* [5], *P. pisi* [2], *P. primulae, P. ribicola* [3], *P. ribes* [2], *P. sesami* [2], and *P. woodsii* [2].

Of the phytopathogenic pseudomonads tested, there was growth in the tartrate medium only of *Pseudomonas aceris, P. alliicola, P. aptata, P. barkeri, P. caryophylli, P. cichori, P. marginata, P. mellea, P. pisi, P. tabaci, P. viridilivida,* and *P. washingtonia.*

In the tyrosine medium, about half of the tested phytopathogenic *Pseudomonas* species produces a definite brown pigment, which more often turns an olive-green when it combines with the water-soluble green pigment so frequently produced by members of this group. These tyrosinase-positive species are: *P. alliicola, P. angulata, P. apii, P. barkeri, P. berberidis, P. coronafaciens, P. delphinii, P. maculicola, P. papulans, P. primulae, P. ribicola, P. savastanoi* var. *fraxini, P. tabaci,* and *P. viridiflava.* Four species, *Pseudomonas pisi, P. savastanoi, P. sesami,* and *P. tomato,* also produced this coloration but to a lesser extent. The other pseudomonads failed to produce the red-to-brown coloration under the conditions specified in the section on "Methods"; although many of these cultures made brown pigment from tyrosine under other testing conditions (18). It is noteworthy that *P. caryophylli* produces its typical brown cell-pigment regardless of the addition of tyrosine to the medium.

The common saprophyte of this genus, *Pseudomonas fluorescens,* is usually reported as lipolytic, but most of the phytopathogens are "not lipolytic" by the spirit blue agar method.[5] The reaction of some of these has been determined (22), and aids in classifying these bacteria.

[4] Numerals in brackets indicate the number of isolates of a particular species which was used by us, when more than one was available.

[5] "Lipolytic" is used here in the limited sense in which it was employed by Starr and Burkholder (22); that is, on the basis of the blue reaction shown in the spirit blue–cottonseed oil–agar method. A "lipolytic" reaction on this medium probably indicates hydrolysis of the cottonseed oil, with liberation and accumulation of certain fatty acids that cause the spirit blue indicator to become blue. Some fat-hydrolyzing, oxidative

The *Pseudomonas* species which were tested do not liquefy the pectate medium. This is especially interesting in the case of *P. alliicola,* that causes a soft-rot of onion. Presumably this species possesses other pectic enzymes than the one which attacks the pectate of this medium, or it is able to induce the rot by some other means.

The NaCl-tolerance of phytopathogenic pseudomonads may, in some cases, be used as a determinative character, although generally the differences between species are not great. Some species (e.g., *P. berberidis, P. solanacearum, P. viburni,* and *P. woodsii*) are characteristically inhibited by as little as 2 per cent NaCl. On the other hand, two cultures, one each of *P. marginata* and *P. viridilivida,* required 10 per cent salt to completely inhibit growth; these data, however, should be checked with additional isolates.

There are but few other properties that can be used as determinative characters in this group. Milk, as stated above, always turns alkaline, but certain species clear it, whereas others do not. The production of a water-soluble green pigment in certain media is a fairly common, but by no means universal, property. Non-pigment-forming strains have been reported in species ordinarily forming the pigment. Like all identifications based on pigmentation, there are limitations to its use.

XANTHOMONAS

The genus. Members of the genus *Xanthomonas* (9) are readily recognized by their abundant, slimy, yellow growth on sugar-containing media. The yellow pigment is a water-insoluble material having the properties of a carotenoid and a maximum absorption in ethanol at about 445 mμ (19). All xanthomonads are aerobic, gram-negative, non-spore-forming, small rods, motile with a single polar flagellum. They are proteolytic, liquefying gelatin and peptonizing milk with, as a rule, the deposition of tyrosine crystals in the latter. They form hydrogen sulfide, but not indol; most of them give the "lipolytic" reaction[5] on spirit blue-cottonseed oil agar (22); they form acid, but not gas, from a variety of carbohydrates; their minimal nutritive requirements are relatively simple (20), yet they are unable to use asparagine as the sole source of both carbon and nitrogen (23); they are not particularly tolerant of sodium chloride, the growth of many of them being retarded by only 2 or 3 per cent of NaCl, and all of them fail to grow in a 5 per cent NaCl medium; they remain viable on potato-dextrose agar or on yeast extract-dextrose-$CaCO_3$ agar, but die off early when cultured on sugar-free beef-peptone or on yeast extract agar media.

The species. Unequivocal identification, by laboratory procedures, of all *Xanthomonas* species is as yet an impossibility. From published descrip-

bacteria may so completely decompose the cottonseed oil, that the fatty acids are degraded beyond the point where they can effect a permanent color change of the indicator. Such a thorough decomposition of cottonseed oil would, perhaps, not be recognized by the spirit blue–agar technique, and fat-splitting organisms capable of this type of decomposition might be classed as not "lipolytic" by the spirit blue–agar procedure.

tions, many of the named species seem identical in cultural characteristics and appear to differ only in that they had been isolated from some particular host. Since the cross-inoculation tests reported in the original descriptions of many of these species have been narrow in scope or entirely lacking and later tests are scattered through the literature, the idea is prevalent that a great number of synonyms exist in this genus. Probably, there is some synonymy; however, at least one fairly comprehensive series of cross-inoculation experiments has been carried out (24, 25), wherein 17 species and varieties of *Xanthomonas* were shown to be completely specific in their pathogenicity.

Let us see in how far bacteriological tests help distinguish *Xanthomonas* isolates which originate from different hosts. The ability to hydrolyze starch divides the genus into two groups. A few of the species are not lipolytic[5] on spirit blue-cottonseed oil agar. A recent study of the nutrition of this genus (20) shows that at least three species have distinctive nutritive requirements which, after more study and when used with suitable precautions, might be employed as determinative characters.

Some exceptional xanthomonads reduce nitrates to nitrites; in our own experience[6] nitrate-reduction to nitrite was observed for the 2 isolates of *Xanthomonas barbareae*, for 2 of the 4 tested *X. begoniae*, for the single cultures of *X. cucurbitae* and *X. lactucae-scariolae*, for the 2 *X. papavericola*, and for about half of the *X. geranii* and *X. pelargonii* isolates. In addition, 2 species which we do not believe belong in this genus, *X. beticola* and *X. rubrilineans*, are strong nitrate-reducers. Because of the existence in some species of strains differing in their reactions to this test, it should be used with caution for determinative purposes. Furthermore, as noted above we do not consider all our results necessarily valid for taxonomic purposes because of the limited number of isolates of some species. We do wish to indicate that only a minority of *Xanthomonas* species are able to reduce nitrates to nitrites, and that strain differences are sometimes seen.

Our trials with the pectate medium show that about a half of the tested *Xanthomonas* species was able to liquify this medium; viz., *X. barbareae*, *X. campestris*, *X. carotae*, *X. geranii*, *X. incanae*, *X. lespedezae*, *X. manihotis*, *X. papavericola*, *X. pelargonii*, *X. phaseoli* var. *sojensis*, *X. taraxaci* (weak or doubtful), *X. vascularum*, *X. vesicatoria* from tomato, *X. vesicatoria* var. *raphani*, and *X. vignicola*. Generally, the pectate was liquified more slowly by the xanthomonads than by soft-rot *Erwinia* species. The ability to liquefy pectate was not correlated in any apparent manner with a capacity to digest starch, nor with any particular pattern of disease symptomatology.

[6] The following *Xanthomonas* cultures were used in most of our investigations (the figure in brackets indicates the number of isolates generally used, when more than one was tested): *Xanthomonas barbareae* [2]; *X. begoniae* [2]; *X. beticola* [2]; *X. campestris* [5]; *X. campestris* var. *armoraciae*; *X. carotae*; *X. corylina* [3]; *X. cucurbitae*; *X. geranii* [3]; *X. hederae*; *X. incanae*; *X. juglandis* [4]; *X. lespedezae* [2]; *X. malvacearum* [2]; *X. manihotis* [7]; *X. papavericola* [2]; *X. pelargonii* [2]; *X. phaseoli* var. *fuscans* [3]; *X. phaseoli* var. *sojensis* [4]; *X. pruni* [4]; *X. rubrilineans*; *X. taraxaci*; *X. translucens* including 5 *formae speciales* [8]; *X. vascularum*; *X. vesicatoria* [4] from tomato and [6] from pepper; *X. vesicatoria* var. *raphani*; *X. vignicola* [2]; and *X. vitians*.

Only three of the *Xanthomonas* species which we tested are characteristically tyrosinase-positive, producing in tyrosine-containing media red colors which soon become brown. *X. phaseoli* var. *fuscans* is most active in this respect; *X. geranii* and *X. pelargonii* also give this reaction, whereas the other *Xanthomonas* species are incapable of producing the red-to-brown coloration under the conditions specified in the section on "Methods." However, some additional cultures do produce red-to-brown reactions under other conditions (18).

The tolerance of the genus *Xanthomonas* for sodium chloride is not great. As noted above, no xanthomonad grows in 5 per cent NaCl broth.[7] Several species grow in 4 per cent salt broth; many grow in 3 per cent. *X. pruni* is probably the most sensitive species, the growth being retarded by 1 per cent NaCl and inhibited completely by 2 per cent. This characteristic seems stable enough for determinative purposes; indeed, the uniformity of results for the replicate isolates of a given species was rather surprising in view of the diverse origins of the cultures.

Another promising approach to this problem is provided by the recent work of Elrod and Braun (10) with agglutination reactions, using antigens low in slime concentration. They were able to group *Xanthomonas* species into several categories, thus aiding identification on some subgeneric level.

SUMMARY

The majority of phytopathogenic bacteria are now grouped in the genera *Pseudomonas* and *Xanthomonas*. The descriptions of many of the species, unfortunately, have offered mainly characteristics of use only in identifying the genus to which they belong; consequently it is often impossible to key out, by means of the usual bacteriological procedures, the species of these two genera. In the present paper, we have endeavored to point out those cultural and biochemical properties that are useful in characterizing the genera of phytopathogenic *Pseudomonas* and *Xanthomonas*. For determining the species, there are presented reactions that have proved useful in the past, as well as experimental work on the utilization of pectate and tartrate, production of a brown pigment from tyrosine, and the ability to grow in the presence of various concentrations of sodium chloride.

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[7] Incidentally, this characteristic affords another method for distinguishing the corn-wilt bacterium, *Bacterium stewartii*, from xanthomonads; the corn-wilt organism generally grows in the presence of 6 or 7 per cent of salt, at which level no *Xanthomonas* species develops.

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PHYTOPATHOLOGICAL NOTES

Pellicularia Target Spot Leaf Disease of Kenaf and Roselle.[1]—Kenaf, *Hibiscus cannabinus* L., was introduced to the Tingo María zone of Peru in 1943 as one of the first fiber plants brought in for adaptability trial by the Estación Experimental Agrícola de Tingo María (now named Estación Central de Colonización en Tingo María). The 1943 planting and subsequent plantings during 1944 and 1945 were small and were made primarily for obtaining seed to maintain the selection. In 1946 larger scale trials were started in response to the need for a domestic grown fiber for bags. The 1946 plantings were successful and in general disease-free. The success of these plantings resulted in an area increase in 1947 to allow sufficient production of fiber for a commercial test of its uses in Peru. No disease problems were apparent until just before harvest. At that time small, occasionally zonate leaf spots characterized by a red halo were observed. Within a few weeks the infestation was general through the planting and during the closing weeks of the wet season (April) the large zonate spots (Fig. 1, A, and B) were present on almost every leaf. On the upper side of the leaf (Fig. 1, A) the spots have the typical zonate formation bordered by a brilliant red halo. On the under side of the leaf the zonate character is less distinct and the red halo almost completely lacking (Fig. 1, B). Characteristically a cobwebby growth of mycelium is found 5–10 mm. in advance of the necrotic zonate areas. Observations indicate that infections occur primarily on young leaves and develop as the leaves grow.

Tissue isolations from advancing margins of the leaf spots uniformly yield a species of *Rhizoctonia*. On the older portions of the spots, especially against the veins, fruiting of a species of *Pellicularia* may occasionally be found. This species of *Pellicularia* has spores averaging $3.4–4.3 \times 6.8–8.5\mu$ and under present concepts of this genus is referable to *P. filamentosa* (Pat.) Rogers.[2]

The first introductions of roselle, *Hibiscus sabdariffa* L., were made in 1947 about the time the disease had become widespread on the kenaf. The roselle was not planted near the kenaf but was in close proximity to nursery beds of rubber heavily infected with a similar leaf spot also caused by a strain of *Pellicularia filamentosa*.[3, 4] These plants were almost at once attacked by *P. filamentosa*, which causes a leaf spot on them, and were

[1] A contribution from the Estación Central de Colonización en Tingo María, Peru, a technical agricultural service organization for the Orient of Peru, operated jointly by the Dirección de Colonización y Asuntos Orientales, Ministry of Agriculture of Peru and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the U. S. Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Peruvian Government.

[2] Rogers, Donald P. The genus Pellicularia (Thelephoraceae) Farlowia 1: 95–118. 1943.

[3] Kotila, John E. Rhizoctonia foliage disease of Hevea brasiliensis. Phytopath. 35: 739–741. 1945.

[4] Lorenz, Rolland C. A new leaf disease of Hevea in Peru. Journal of Forestry (In press).

severely retarded in growth. Microscopically the Pellicularia stage appears identical with that on kenaf and is indistinguishable from the Rhizoctonia stage isolated from the leaf tissue. Symptoms of the disease on the leaves are almost identical (Fig. 1, B and C). Rhizoctonia has been reported on roselle in El Salvador[5] causing stem canker but is probably not a manifestation of this same disease. Insofar as can be determined, the diseases herein reported have not previously been described either in Peru or elsewhere on kenaf or roselle.

Okra, *H. esculentus* L., another member of the genus *Hibiscus*, is grown in close proximity to the kenaf but thus far has remained free of the disease.

In addition to the leaf disease on rubber previously mentioned, web-blight

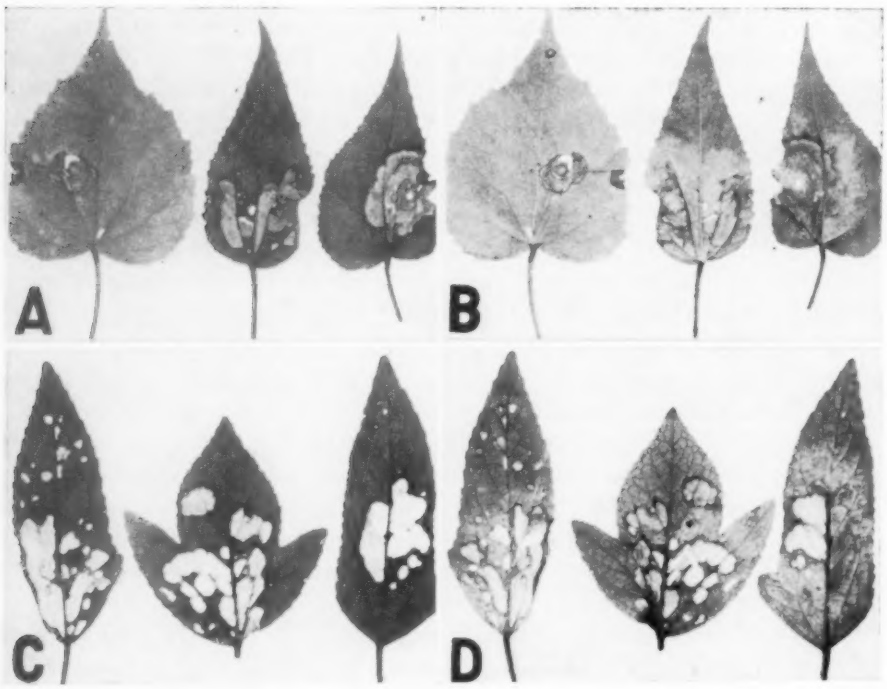

FIG. 1. A. Pellicularia target spot disease on upper surface of leaves of kenaf. B. Lower surfaces of same leaves showing cobwebby mycelial growth. C. Similar disease on upper surface of roselle leaves. D. Lower surface of same leaves showing mycelial growth.

of beans,[6] heretofore not observed in this zone, made its appearance in the experimental fields of the Station about the same time as the disease on kenaf. This disease is also caused by *Pellicularia filamentosa*.[2]

Artificial inoculation with spores has not been possible since thus far none have been produced in culture and naturally produced spores are not

[5] Reinking, O. A. Diseases of Roselle fiber plants in El Salvador. U. S. Bureau of Plant Industry, Plant Disease Reporter 29: 411–414. 1945.

[6] Weber, George F. Web-blight, a disease of beans caused by Corticium microsclerotia. Phytopath. 29: 559–575. 1939.

sufficiently abundant to allow their use. It has, however, been possible to use infected leaves and agar cultures of the *Rhizoctonia* stage for cross inoculations. In these cross inoculation trials the strain of *Pellicularia filamentosa* from beans was included since the leaf symptoms were similar, but primarily as a fungus control on the inoculation method.

Leaves of roselle and rubber with active lesions were placed in contact with young leaves of these hosts and kenaf, and a disease with similar symptoms developed on rubber and roselle but not on kenaf. In another trial, a leaf spot developed on roselle, kenaf, and rubber when infected material from rubber was used as the source of inoculum.

Agar cultures of the Rhizoctonia stages from the disease on kenaf, roselle, beans, and rubber were subsequently used to inoculate 4 leaves each by placing agar blocks of mycelium between the lower surfaces of two leaves and clamping them together. With this method the isolate from kenaf successfully produced the disease in 4 of the 4 inoculations on rubber, in 4 of the 4 inoculations on kenaf, and in 2 of the 4 inoculations on roselle. The isolate from roselle produced the disease only on 4 of the 4 leaves of roselle, the rubber isolate produced the disease only on rubber. The isolate from beans produced typical target spots of more virulent type than the natural spots in all 4 of the inoculated leaves of kenaf, roselle, and rubber. Although not entirely conclusive, these preliminary inoculations indicate that the target spot leaf diseases of rubber, kenaf, roselle, and web-blight of beans are apparently all manifestations of disease produced by the same or slightly different strains of *Pellicularia filamentosa.* Check inoculations on all hosts using sterile agar blocks resulted in no infections and no injury to the leaves.

The disease on the present kenaf crop does not appear to have seriously affected fiber production. Infected roselle plants developed much slower than healthy individuals. During the dry season the disease is still in evidence but its intensity is much reduced. Roselle and kenaf, if grown during the wet season, may need spray protection at least during the first stages of growth.—BOWEN S. CRANDALL, Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture, and Technical Chief, Department of Plant Pathology and Entomology, Estación Central de Colonización en Tingo María, Peru.

Physalis angulata, a Test Plant for the Potato Leafroll Virus.—A study of potato leafroll was handicapped by the fact that a suitable test plant was not available which could be grown easily and which would show distinct symptoms of the disease.

The potato plant is not suitable for studying some phases of the leafroll problem. It is propagated vegetatively and hence each tuber used for the experiments must be checked to ascertain that it is not already harboring the virus. Potato plants are relatively large and occupy much valuable space in the greenhouse. The symptoms of leafroll often are

slow to develop or are masked, and often it is necessary to grow the progeny of the test plants the following year before the results of the inoculation studies can be obtained.

These handicaps in studying the leafroll virus were eliminated by using *Physalis angulata* L. as the test plant. This plant is propagated from true seed which readily germinate without requiring a long rest period. It is rather small and easily grown in small pots in the greenhouse and it is a good host plant for the different potato aphids. Furthermore, the symptoms of the leafroll disease become apparent on the the test plant about 10 days after the plants have been inoculated. This plant shows distinct symptoms of the leafroll disease when inoculated

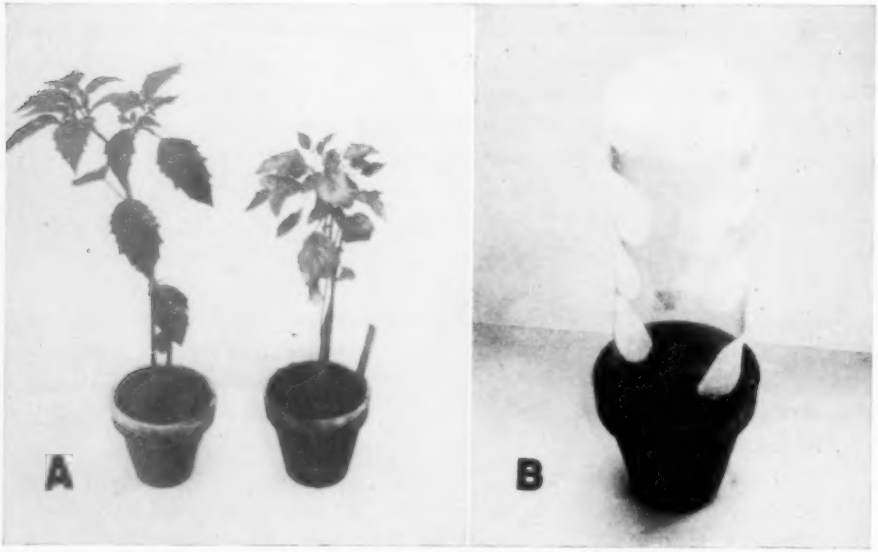

FIG. 1. A. Left, healthy *Physalis angulata* control. Right, *P. angulata* with leafroll transmitted by *Myzus persicae* (Sulz.) from diseased Katahdin potato plant. B. Small plant covered with plastic cage after being inoculated with aphids.

with viruliferous aphids, the infected plants becoming dwarfed with chlorotic and rolled leaves (Fig. 1, A).

The plants used for the leafroll studies are grown in 4–inch pots in the greenhouse and are inoculated with aphids when from 2 to 3 inches high. The viruliferous aphids are transferred to the test plants with a camel's-hair brush. Plastic cages are fitted over the inoculated plants and the aphids are allowed to feed about 5 days (Fig. 1, B). The cages are then removed and the aphids killed by fumigation. The plants are examined for leafroll symptoms from 10 to 20 days after they are inoculated.

The use of *Physalis angulata* as a test plant has materially shortened the time necessary for making inoculation experiments with the leafroll virus and has been useful in studying certain phases of the aphid-leaf-

roll relationship. The studies indicate that although certain non-solanaceous wild plants normally serve as breeding hosts for the potato aphids, they apparently do not harbor the leafroll virus. Infested potato plants in the growers' seed stocks appear to be the chief source of infection.

The studies confirmed the data obtained with the potato, that the peach aphid, *Myzus persicae* (Sulz.), is the chief vector of potato leafroll in Maine. The potato aphid, *Macrosiphum solanifolii* (Ashm.), and the buckthorn aphid, *Aphis abbreviata* Patch, did not readily transmit the leafroll virus to *Physalis angulata*.

It was shown that leafroll can be transmitted by a single viruliferous peach aphid inoculating the Physalis plants. However, often no infection resulted when single aphids were used. About 75 per cent of the plants became infected when five viruliferous aphids were used to inoculate each Physalis plant.

The virus was transmitted by all of the nymphal stages of the peach aphid, and the younger aphids (first and second instars) transmitted the disease as readily as did the mature apterous aphids.

Winged aphids are very numerous in Maine during late summer when they are migrating from the potato fields. It was assumed that these aphids are very important factors in the dissemination of the leafroll virus. The data showed that less than 0.1 per cent of the winged migrating peach aphids were viruliferous when tested on *Physalis angulata*. About 24 per cent of the winged peach aphids were viruliferous when it was known they had fed on potato plants which had the leafroll disease.

The leafroll virus was readily transmitted from infected Physalis plants to healthy Katahdin potato plants by the peach aphid, and typical symptoms of the disease resulted.—CHARLES HOVEY, formerly of the Maine Agricultural Experiment Station, and REINER BONDE, Maine Agricultural Experiment Station, Orono, Maine.

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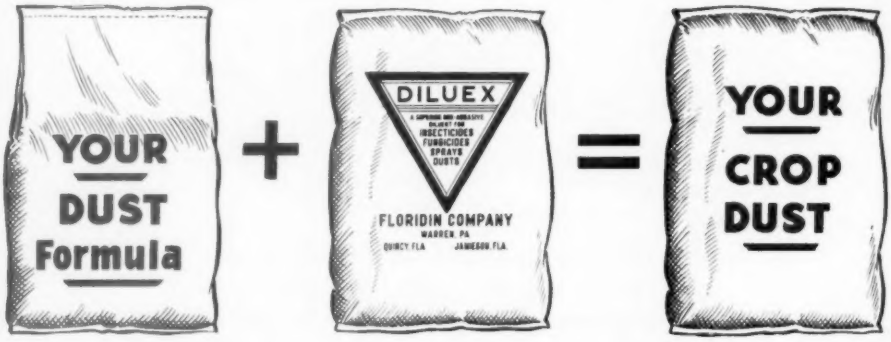

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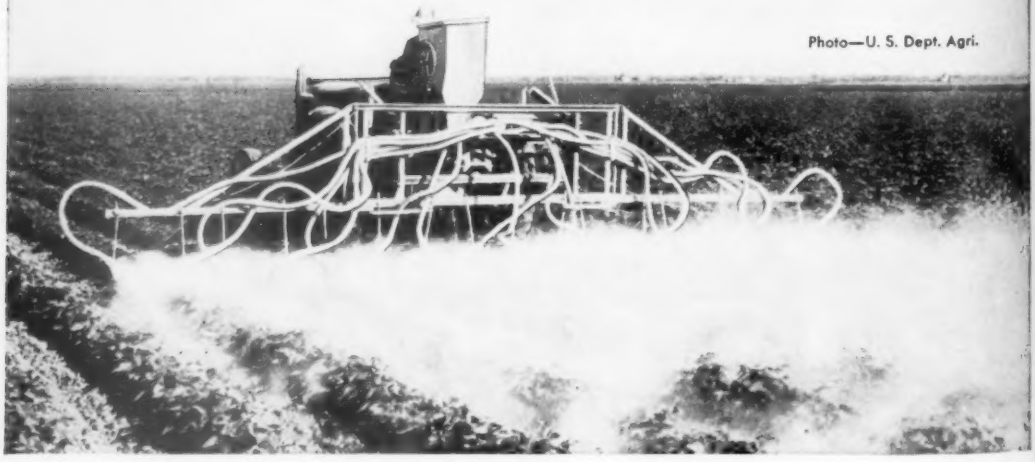

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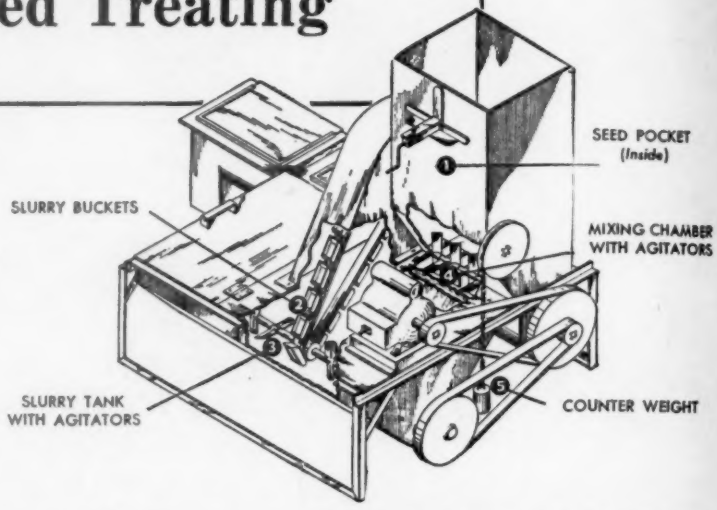

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